

**Trophic Transfer of Inorganic Selenium Species through
Representative Freshwater Food Chains**

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ABSTRACT

In recent decades, there has been growing interest in the toxicodynamics of the natural element selenium (Se) and its most commonly encountered chemical species, with a notable focus on freshwater ecosystems that have shown toxicological sensitivity. Exposure to elevated Se has uncovered an unusually narrow threshold between its biological necessity and toxicity, particularly among the most sensitive taxa, including oviparous vertebrates for which exposure to excess Se can lead to teratogenicity and reproductive failure. Uncertainties persist relating to the trophic transfer of dietary Se to higher organisms, originating from the bioconcentration of aqueous-borne chemical species and their biotransformation at the base of the food web. As a result, research has been ongoing to discern the influence of naturally variable biogeochemical characteristics on the enrichment, transformation, and trophic transfer of Se into the taxa of greatest concern.

The purpose of this thesis was to contribute to the current understanding of Se trophic dynamics by evaluating Se trophic transfer to higher consumer species, originating from dissolved inorganic chemical species of concern. Diets of field-collected biofilms showing a diversity of community compositions were exposed to control conditions ($0.3 \mu\text{g Se L}^{-1}$) and to graded concentrations of selenite (SeIV) and selenate (SeVI) (5 and $25 \mu\text{g Se L}^{-1}$), respectively, and fed to a primary consumer species common to Canadian freshwater ecosystems, *Hyallela azteca*. Generally, SeIV was transferred through trophic levels following a concentration-dependent relationship, with the greatest divergence among tissue Se residues occurring between the invertebrates exposed to different field-collected biofilm communities, with trophic transfer factors ranging from $0.15 - 0.51$ among all SeIV exposed treatments. Final mean *H. azteca* tissue Se concentrations ranged from $2.7 - 6.5 \mu\text{g Se g}^{-1} \text{ dw}$ in the low SeIV treatment and $7.3 - 18 \mu\text{g Se g}^{-1} \text{ dw}$ in the high SeIV treatment. Uptake of SeVI into the invertebrate tissues was not significantly different from the

control treatment. Differences in bioconcentration and trophic transfer between chemical species and treatments appeared to vary as a function of differences in primary producer biomass and ionic competition for uptake.

For the purpose of further evaluating Se trophic transfer to a more sensitive oviparous species at a higher trophic level, an environmentally relevant freshwater food chain was created. A probable exposure scenario was simulated through aqueous SeIV exposure of green alga *Stichococcus bacillarus* that was fed to *H. azteca*, which then served as the diet for a common Canadian freshwater secondary consumer species, the fathead minnow (*Pimephales promelas*). Three *H. azteca* dietary treatments ($1.6 \mu\text{g Se g}^{-1} \text{ dw}$ [control], $6.9 \mu\text{g Se g}^{-1} \text{ dw}$, and $19 \mu\text{g Se g}^{-1} \text{ dw}$) were collected and fed to the fish species in a partial lifecycle reproductive assay. Final muscle, gonad, and liver Se concentrations showed concentration-dependent increases with greatest concentrations of 4.5 ± 1.1 , 16 ± 1.8 and $17 \pm 1.9 \mu\text{g Se g}^{-1} \text{ dw}$, respectively, in adult female fish in the high SeIV treatment. Reproduction was negatively affected by elevated Se exposure, and resulting incidence of fish fry deformities in reared F1 offspring was greatest in the low SeIV treatment ($37 \pm 3.0 \%$). Embryo Se concentrations reached up to 7.5 and $17 \mu\text{g Se g}^{-1} \text{ dw}$ in the low and high SeIV treatments, respectively.

This research showed how bioconcentration and trophic transfer of Se depended both on chemical species and primary producer community composition. Releases of Se into aquatic environments from anthropogenic activity will therefore lead to site-specific differences in Se movement along food chains and subsequent toxicity. These factors should be considerations for the future of Se management and research.

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°C	degrees Celcius
$\mu\text{g g}^{-1}$	micrograms per gram
$\mu\text{g kg}^{-1}$	micrograms per kilogram
$\mu\text{g L}^{-1}$	micrograms per litre
μm	micrometer
μM	micromolar
$\mu\text{S cm}^{-1}$	microsiemens per centimeter
Ω	ohm
AE	assimilation efficiency
ANOVA	analysis of variance
ATRF	Aquatic Toxicology Research Facility
BC MoE	British Columbia Ministry of Environment
CaCO_3	calcium carbonate
cm	centimeter
cm^2	squared centimeters
CPCC	Canadian Phycological Culture Centre
DO	dissolved oxygen
dw	dry weight
EC_{10}	10% effect concentration
EDTA	ethylenediaminetetra-acetic acid
EF	enrichment function
F1	first filial generation
FeS_2	pyrite
g	gram
GSH	glutathione
GSI	gonadosomatic index
GSSG	glutathione disulfide
H_0	null hypothesis
HDPE	high density polyethylene
HNO_3	nitric acid

ICP-MS	inductively coupled plasma mass spectrometry
K	condition factor
K_d	distribution coefficient
kg	kilogram
K-W	Kruskal-Wallis H test
L	litre
LC_{50}	50% lethality concentration
LSI	liversomatic index
Mg	milligram
$mg\ cm^{-2}\cdot^{-1}$	milligram per square centimeter
$mg\ kg^{-1}$	milligram per kilogram
$mg\ L^{-1}$	milligram per litre
mL	millilitre
mm	millimeter
mM	millimolar
Na_2SeO_3	sodium selenite
No_{aq}	no aqueous selenium treatment
PAR	photosynthetically active radiation
PO_4	phosphate
PVC	polyvinyl chloride
RM	repeated measures
RPM	revolutions per minute
Se	selenium
SE	standard error
Se^{2-}	selenide
Se^0	elemental selenium
Se_{aq}	aqueous selenium treatment
SeCys	selenocysteine
SeIV	selenium in +4 oxidation state (selenite)
SeMet	selenomethionine
SeO_3^{2-}	selenite ion

SeO ₄ ²⁻	selenate ion
SeVI	selenium in +6 oxidation state (selenate)
SLD	San Luis Drain
SO ₄	sulfate
TTF	trophic transfer factor
US-EPA	United States Environmental Protection Agency
ww	wet weight

PREFACE

This thesis is organized in manuscript style, with chapters 2 and 3 currently being prepared for publication in peer reviewed journals; as such, there is some repetition of the Introduction and Materials and Methods sections among the different chapters. Chapter 2 will be submitted to *Environmental Science and Technology*, and Chapter 3 will be submitted to *Aquatic Toxicology*. Supplementary information that will be published from Chapters 2 and 3 has been included in Appendix A.

Raes, K.A., Doig, L.E., Markwart, B., Liber, K., Janz, D.M., & Hecker, M. (2020). Selenium oxyanion enrichment in field-collected biofilms and trophic transfer to a primary consumer. *Environ Sci Technol*, (in preparation).

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CHAPTER 1

INTRODUCTION

1.1 Historical background of selenium

Selenium (Se) is a naturally occurring element whose globally growing mobilization has its environmental fate and toxicity under careful examination. Selenium has been recognized as a contaminant of concern to aquatic organisms, particularly egg-laying species, for the better part of eight decades (Ellis et al. 1937); however, its toxic effects remained largely unnoticed until the mid-1970s, when it was discovered that the large-scale combustion of coal at steam-electric generating plants could result in a significant input of the element into aquatic environments (Andren et al. 1975; Kaakinen et al. 1975). Long-term monitoring of Belews Lake and its use as a power plant cooling reservoir, served as the first well-documented occurrence of a multi-species, fish population decline event resulting from elevated Se (Lemly 1985). Findings from this case study established that Se had entered the lake in the return flow from the ash settling basin at concentrations in the range of 150-200 $\mu\text{g Se L}^{-1}$, which resulted in accumulation of the element in benthic organisms and trophic transfer to fishes. Subsequently, dietary toxicity and reproductive failure were observed to varying extents in the resident fish community (Cumbie & Van Horn 1978).

Selenium contamination occurs when anthropogenic processes redistribute sequestered, Se-containing subterranean resources. Such processes include mining activities, fossil fuel

combustion, oil refining, fertilizer production, and agricultural irrigation (Presser et al. 1990). The nearly ubiquitous biological essentiality of Se-containing proteins and the corresponding narrow threshold for its toxicity, particularly in oviparous organisms such as fish and birds, provide the element with unique properties of concern (Lemly 2002). Selenium loading into aquatic ecosystems is likely to continue in the coming years in Canada, due to the ongoing development of mining activities and agricultural irrigation to satisfy the growing global energy and food demand (Mining Association of Canada 2019; Statistics Canada 2017). Inorganic forms of Se released by these activities are efficiently assimilated, biotransformed, and bioaccumulated by aquatic microorganisms (e.g. algae, fungi, bacteria) into organo-Se compounds, which are transferred to higher trophic levels via dietary pathways (Stewart et al. 2004; Janz et al. 2014). Once significant Se contamination has occurred, the succeeding cascade of trophic transfer events can result in major ecosystem disruption (Lemly 1985).

Despite the release of primarily inorganic Se chemical species, selenomethionine (SeMet) is the Se species of most concern. Selenomethionine is regarded as the primary organic Se form identified at the base of the food web, exhibiting the greatest potential for bioaccumulation, and is highly toxic to receptors of concern such as fish (Fan et al. 2002). Because of the increasing recognition of Se biotransformation, bioaccumulation and potential toxicity, the Government of Canada has continued to revise its regulations for the protection of human health and the environment. Selenium was only incorporated into the list of parameters required for effluent characterization and water quality monitoring by the Canadian Metal Mining Effluent Regulations in 2012 (MMER 2002). In 2017, a screening assessment concluded that Se met the criteria for a toxic substance as per the *Canadian Environmental Protection Act, 1999* (Environment and Climate Change Canada [ECCC] 2017). Despite considerable ecotoxicological attention given to

this element over the past decades, significant knowledge gaps persist, particularly related to enrichment at the base of the food web. Difficulties in setting specific Se standards in freshwater environments stem from an inability to predict risk based solely on water quality and the element's dissolved concentration. It has been suggested that due to the influence of spatial and temporal variation of the Se cycle at any ecological site in question, local water quality criteria for Se should reflect site-specific, chemodynamic-altering properties (Lemly 1999). However, the relative importance of biogeochemistry on the uptake and transformation of Se at the base of the aquatic food chain remains poorly understood (Janz et al. 2014). For this reason, this thesis focused on the characterization of inorganic Se uptake into natural biofilm communities and higher trophic model organisms representative of Canadian freshwater ecosystems, to assess its trophic transfer across simulated foodchains using standard conditions.

1.2 Selenium in the aquatic environment

1.2.1 Physicochemical properties of selenium

Physicochemical properties of Se are complex and unique, owing largely to the variety of forms in which this element can exist and the consequent attributes of its allotropes. Selenium is element 34 on the periodic table, possessing an atomic weight of 78.96 u. It belongs to the chalcogen (VIA) group, sharing fundamental properties with other lighter group elements, oxygen and sulphur, and heavier elements, tellurium and polonium. Selenium occurs naturally in four oxidation states: selenide (Se^{2-}), elemental Se (Se^0), selenite (Se^{4+}), and selenate (Se^{6+}). Selenium is generally classified as a non-metal, though the element has several allotropes that display borderline metalloid behaviour. Unlike metals or transition metals, which typically form cations in aqueous solution, Se is hydrolyzed to form inorganic oxyanions including selenite (SeO_3^{2-} ; SeIV) and selenate (SeO_4^{2-} ; SeVI) under oxidizing conditions. These oxyanions generally show an

increased solubility and mobility with increasing pH, in contrast to the behaviour of metals, and comprise the majority of dissolved Se present in the aquatic environment from anthropogenic sources (Presser & Ohlendorf 1987; Maher et al. 2010; Shaw et al. 2011). In general, these more soluble and mobile forms of Se are dominant under aerobic and alkaline conditions, such as in natural water bodies, while elemental Se and selenides are not soluble in water and tend to partition to sediments. Organic forms of Se such as selenomethionine (SeMet) and selenocysteine (SeCys), and volatile dimethylselenide have also been detected in the aquatic environment (Simmons & Wallschläger 2005).

1.2.2 Environmental occurrence of selenium

Selenium is a naturally occurring element, composing a portion of the earth's crust that is susceptible to redistribution by natural and anthropogenic processes. Average concentrations of Se in the earth's crust are typically $<0.5 \text{ mg Se kg}^{-1}$, though areas of enrichment are known to exist in particular geologic formations (Presser et al. 2004a). The global distribution of Se is linked in part to organic-rich depositional marine basins. Major geologic sources of Se include black shale, phosphate rocks, and coal, as well as igneous rock and limestone to a lesser extent (Guun et al. 1976). Natural processes comprise a significant contribution to global Se fluxes, including volcanic activity, weathering of Se-containing sources, wildfires, and volatilization from plants and water bodies (Mosher & Duce 1987); however, environmental Se contamination is often a result of the industrial processing of earth within Se-enriched geological formations (Presser et al. 1990; Muscatello & Janz 2009). In addition to geologic associations, Se is also associated with various sulphide ores of commercial interest, including copper, silver, lead, mercury, and uranium (Wang et al. 1993), including sulphide-associations the Elk River Valley, an area of concern in Canada (Lussier et al. 2003). Selenium in these deposits can occur as both organic and inorganic

species (Yudovich & Ketris 2006), though processing can result in species transformation and transfer between phases (e.g. liquid to solid) (Haygarth 1994). Selenium can be mobilized slowly through natural weathering from host rock matrices, but this process is significantly accelerated by anthropogenic activity, namely by mining activities that expose the ore to oxidation (Phibbs et al. 2011) and agricultural sources (Kausch & Pallud 2013).

Selenium influx into the environment occurs through a number of sources, producing a range of concentrations and chemical species, though water is the primary driver for anthropogenically sourced Se into aquatic environments (Maher et al. 2010). Selenium commonly occurs as a mixture of different chemical forms in surface waters, though SeIV and SeVI are predominant. In shallow aquatic bottom sediments, which commonly exhibit reducing conditions, Se of different oxidation states are rapidly reduced to selenides and elemental Se (Zawislanski & McGrath 1998). Selenides and elemental Se are not characteristically bioavailable primarily due to their very low solubility; however, they can be transformed into bioavailable species under oxidizing conditions or by uptake and biotransformation by microorganisms (Lemly 1999). Though inorganic Se species are dominant in the aqueous environment, the transfer of organic species through the food web has been hypothesized to be largely responsible for governing the element's toxicity to higher animals (Presser & Luoma 2009). However, the understanding of Se biotransformation is limited, particularly in regard to aquatic primary producers, which are believed to drive Se biogeochemical cycling in aquatic environments (Stewart et al. 2010).

The non-metallic behaviour of Se may govern a significant portion of its geochemical nature; nonetheless, biologically mediated reactions dominate the routes of metabolic activity in higher organisms. The total concentration of Se in a particular environmental compartment has limited predictive capacity when considering resulting toxicity because the fate and bioavailability

of Se are governed primarily by its speciation and the potential for biotransformation in the receiving environment (Chapman et al. 2010). Selenium tissue residues at contaminated sites differ among taxa despite similar trophic levels (Stewart et al. 2004), highlighting the need for complimentary understanding of both geochemical and biological interactions at play in Se enrichment and trophic transfer.

1.3 Physiological role of selenium

1.3.1 Biological essentiality of selenium

Biological exposure to Se has a paradoxical history, with farmed livestock often requiring supplementation, while aquatic oviparous vertebrates have encountered toxicity from high environmental Se concentrations. Selenium was first recognized as a biologically essential element in 1957 (Mayland 1994), which holds true for all living organisms with the exception of higher plants and yeasts (Hesketh 2008). It is an essential micronutrient and a key component required for normal growth and development, present in all three major forms of life (bacteria, archaea, and eukaryotes) (Gladyshev & Kryukov 2001). The physiological essentiality and eventual toxicity of Se can largely be credited to the three biological states in which the element can exist in living organisms, which include: 1) trace concentrations required for normal growth and development, 2) moderate concentrations that can be stored while maintaining homeostatic function, or 3) elevated concentrations that result in toxic effects (Eisler 2000). For humans, $0.6 \mu\text{g Se kg}^{-1}$ is the recommended daily intake, functioning in antioxidant and immune-strengthening processes (Papp et al. 2007). The element has also gained favour as an antitumorigenic agent, with greater supplementation increasingly defended as a cancer prevention strategy (Rayman 2002). Recommended intake is variable among taxa, with limited information available for optimal conditions in some groups (e.g. aquatic invertebrates) (Stewart et al. 2010).

1.3.2 Selenoproteins

The metabolism of Se is diverse and highly specific, owing to the complex routes of transformation involved in the formation of Se-containing proteins. Three broad categories of Se-containing proteins have been classified: 1) those with nonspecifically incorporated Se (e.g. SeMet), 2) Se-specific bound proteins (e.g. selenophosphate synthetase), and 3) enzymes with SeCys incorporated into their active site (e.g. iodothyronine 5'-deiodinase) (Patching & Gardiner 1999). Many Se-containing proteins remain unidentified and their functions undetermined; therefore, many mechanisms of metabolism and potential toxic action are undefined (Lazard et al. 2017). Common families of selenoproteins with described physiological functions include glutathione peroxidases, thioredoxin reductases, iodothyronine deiodinases, and selenophosphate synthetases, which are all known to have oxidoreductase functions, catalyzing the transfer of electrons in fundamental physiological processes (e.g. DNA synthesis) (Papp et al. 2007). Selenoproteins maintain antioxidant defense systems, a mechanism that has also proven dangerous when Se exposure becomes elevated. The propagation of oxidative stress in Se-exposed organisms appears to be critically impacted by an interaction with glutathione (GSH) (Spallholz et al. 2004). A lowered ratio of reduced GSH to oxidized glutathione disulfide (GSSG), and increased indices of oxidative stress have been observed in birds and fish exposed to elevated Se (Hoffman 2002; Holm 2002). In vertebrates, Se can be incorporated into all three aforementioned forms of Se-containing proteins, though fish in particular possess the most abundant selenoproteome with 30 recognized selenoproteins (Castellano et al. 2005), compared to 25 selenoproteins identified in the human selenoproteome (Kryukov et al. 2003).

Selenium chemical species consumed in the diet are considered the predominant pathway for uptake, where the element is readily absorbed from an animal's intestine, and stored as SeMet

or SeCys in proteins or as SeIV complexed to proteins (Lobinski et al. 2000; Dumont et al. 2006). Relative to their sulphur analogues, methionine and cysteine, the formation of SeMet and SeCys appear to use sulphur pathway enzymes and depend on relative concentrations of Se in the system (Allan et al. 1999). In studies focused on elevated Se exposure, organic SeMet has been reported as the predominant Se species among living organisms despite inorganic dominance in abiotic compartments (Janz et al. 2014). This incongruence exists due to conversion of inorganic Se species to organo-Se compounds by the primary producer trophic step following its bioconcentration (Doucette et al. 1987; Riedel et al. 1996). Animals are able to synthesize SeCys from accumulated SeMet; however, excess SeMet is efficiently incorporated during protein synthesis by non-specific substitution of methionine, resulting in negative implications for protein dysfunction (Schrauzer 2000).

Incorporation of SeMet into yolk proteins of oviparous vertebrates via maternal transfer is a fundamental pathway driving teratogenicity in impacted embryos (Woock, et al. 1987; Schultz & Hermanutz 1990). Vitellogenin is a sulphur-containing primary egg yolk precursor, synthesized in the liver and incorporated into developing ovarian follicles through transportation in the blood (Kime et al. 1999). Selenium residues accumulate in the eggs and embryos of adversely impacted oviparous vertebrates (Roe et al. 2004; Holm et al. 2005; Covington et al. 2018), and Se bound to vitellogenin has been observed in fish, showing how the protein can act as a vehicle for Se transport into offspring tissues (Kroll & Doroshov 1991). The relative deposition of vitellogenin into developing oocytes is dependent on the reproductive strategy of the organism in question, the timing and duration of oogenesis being highly variable among animal species resulting in variable Se incorporation (Rinchard & Kestemont 2005).

1.3.3 Mechanism of selenium toxicity to aquatic organisms

The bioavailability, metabolism, and subsequent distribution of Se within organisms is dependent on its speciation, route of entry, and the organism's physiology. Identification of the source of Se accumulation is fundamental for the interpretation of contamination in affected organisms. There have been discrepancies in the literature in regard to the adverse effects in fish from Se exposure (Hilton et al. 1980; Hunn et al. 1987; Ogle & Knight 1989). This is likely due in large part to differences in species, life stage, exposure route, and other factors among studies, the true cause of toxicity likely resulting from a combination of factors. In the most sensitive life stage of organisms to Se toxicity, teratogenesis has been a consistent bioindicator of Se exposure (Ohlendorf et al. 1986). Elevated Se concentrations in adult liver, kidney, ovaries, and testes have also been linked with pathological changes adversely affecting those tissues as well as lowering hematocrit and condition factors (Sorensen & Bauer 1983; Wiseman et al. 2011; Zee et al. 2016).

A primary mechanism by which Se teratotoxicity occurs may be through its propensity to substitute for sulphur (Lemly 1997). Due to biochemical similarities between Se and sulphur (e.g. identical outer shell electron configurations in their respective ground energy states), Se can follow the metabolic pathways of sulphur at elevated levels (Sors et al. 2005). Sulphur species and sulphur-containing biomolecules occupy a foremost position in metabolism. The widespread presence, diversity, and importance of bioactive sulphur molecules in living organisms can be attributed to the evolution of life on Earth, where the element was predominant during the origin of life. This theory has been strengthened by the occurrence of highly conserved sulphur metabolism pathways in bacterial species, and the existence of sulphide-producing archaea species, both examples of anaerobic respiration (Palego et al. 2015). Sulphur biomolecules are responsible for a substantial number of important functions in all living organisms, including but not limited

to DNA methylation and repair, regulation of gene expression, protein synthesis, metal transport, and free radical scavenging (Leustek et al. 2000). The biochemical similarity of Se to sulphur predicates widespread opportunistic sulphur-pathway annexation by Se, should its own metabolic pathways be saturated by high concentrations. Substitution for sulphur is most damaging during protein synthesis, which occurs in the midst of embryo organogenesis (Janz et al. 2010). The normal tertiary structure of proteins depends on the formation of sulphur-sulphur linkages, so substitution of Se for sulphur during protein synthesis inevitably results in misfolded and dysfunctional proteins (Diplock & Hoekstra 1976).

Oxidative stress may be an initiating event for teratogenicity and embryo mortality caused by several contaminants, including Se (Spallholz & Hoffman 2002). Evidence supporting the role of oxidative damage from elevated Se during the initiating event in embryo mortality and teratogenicity has been growing (Palace et al. 2004; Kupsco & Schlenk 2016). The propagation of oxidative stress in Se exposed organisms appears to be critically influenced by an interaction with glutathione (GSH) (Spallholz et al. 2004). Acting with glutathione peroxidase, GSH is an intracellular antioxidant that exhibits significant reducing power in the maintenance of antioxidant enzyme systems. A lowered ratio of reduced GSH to GSSG, and increased indices of oxidative cell damage have been observed in birds exposed to Se (Hoffman 2002). The increased ratio of GSSG to GSH in the presence of elevated Se occurs alongside increased hydroperoxides and increased hepatic lipid peroxidation (Hoffman et al. 1989). Selenomethionine is the predominant form of Se in the embryos of oviparous vertebrates, and is not itself highly reactive with GSH, counterintuitive for an oxidative stress mechanism of toxicity (Spallholz et al. 2001). However, *in vivo* metabolism of SeMet and SeCys could be responsible for potentiating oxidative stress, through concentration-dependent incorporation of elevated Se species and successive enzymatic

cleavage into more reactive forms of Se, including methylselenol (Palace et al. 2004). As the cycling of Se species within living cells has become more clear, evidence of significant reactive oxygen species production through the redox cycling of Se-compounds into hydrogen selenide and selenols is implicated in toxic responses (Lazard et al. 2017). Resulting oxidized selenols also have the capacity to react with protein-thiols, forming selenylsulfide bridges and catalyzing the formation of disulfide bridges, unwanted bonds that can alter protein function or cause protein aggregations (Lazard et al. 2017).

1.4 Bioaccumulation and trophic transfer of selenium in aquatic environments

1.4.1 Uptake of selenium by primary producers

The fundamental basis for understanding Se bioaccumulation and trophic transfer in aquatic ecosystems can be described by two forming principles. First, uptake of Se at the base of the aquatic food web by primary producers (e.g. algae and macrophytes) and microorganisms (e.g. bacteria and fungi) comprises the largest contribution to its bioconcentration (Luoma & Presser 2009). Bioconcentration of Se by microorganisms at the base of the food web is highly variable among species, showing concentration by up to 10^6 -fold from the aqueous phase in marine phytoplankton (Baines & Fisher 2001). As a result, diet represents the principal pathway for Se exposure, transfer and toxicity to higher organisms in an aquatic ecosystem (Stewart et al. 2010). A second, less prominent exposure pathway of Se is through the water phase. This can also result in uptake for most aquatic consumers; however, this exposure pathway has been concluded not pose a significant risk in comparison to the dietary pathway (Janz et al. 2010). Due in large part to the conservation of Se's biological essentiality in lower trophic organisms, SeVI and SeMet uptake occurs primarily through active transport by membrane proteins, though uptake of SeIV occurs predominantly by passive absorption (Riedel et al. 1991). The subsequent bioaccumulation of Se

in an ecosystem varies as a function of a number of factors, including species-specific uptake rates, speciation and concentration of the element, water chemistry of the receiving environment, and the water turnover rate (i.e., lentic or lotic) (Reinfelder et al. 1997; Baines & Fisher 2001; Brix et al. 2001; Hillwalker et al. 2006; Franz et al. 2011; Gallego-Gallegos et al. 2013).

To appreciate the mechanism by which Se is effectively incorporated and transferred through biological food chains, one must comprehend the general principles of trophic ecology. Simply, trophic level relationships are described through trophic pyramids, where energy production from an inorganic source is greatest at the base of the food web (Elton 1927). The large energy-pyramid base represents the autotrophs, i.e., the primary producers responsible for photosynthetic energy production, but ultimately support the energy propagation to all higher trophic levels. Adhering to the basic laws of thermodynamics, energy transfer from any one trophic level to the next is constrained by energy lost as heat in any biological process, resulting in the shrinking pyramid structure seen at the higher trophic levels. Ratios of elements in the abiotic environment do not match those of biota, creating imbalances in nutrients in the living and abiotic domains. Trophic ecology and global biogeochemistry are linked because of stoichiometric processes (Schrama et al. 2013).

Generally, uptake of dissolved Se by the primary producer trophic level has been described by a distribution coefficient (K_d), though this term has been losing favour (Stewart et al. 2010). The term K_d has been defined as being a function of the enrichment of Se in the particulate material (e.g. phytoplankton, periphyton, sediment, detritus, etc.) relative to ambient dissolved concentrations (Presser & Luoma 2010); however, this concept of equilibrium partitioning has been argued as misleading for Se because uptake of the element requires energy, not always following linear uptake kinetics, and due to rapid conversion of the element into organic selenides

following its uptake (Stewart et al. 2010). Enrichment functions (EF) have therefore been adopted to better describe the non-linear relationship between Se concentrations in the aqueous-phase and particulate material (DeForest et al. 2017; Markwart et al. 2019). A source of variability in Se bioconcentration ratios is produced by inter-specific variation among taxa (Wrench & Measures 1982; Riedel et al. 1991), where enrichment has been measured exceeding 10^6 in controlled conditions, though differences in enrichment among marine taxa has been observed varying up to five orders of magnitude (Baines & Fisher 2001). Regardless, the initial bioconcentration of Se at the level of the primary producer is largely regarded as the greatest bioaccumulation step in aquatic food chains, and therefore represents a major determinant of Se exposure to higher trophic levels.

1.4.2 Trophic transfer of selenium to higher trophic levels

Understanding the characteristics by which Se bioaccumulation differs among aquatic organisms in diverse environmental conditions is fundamental to the protection of vulnerable organisms. In the context of nutrient dynamics (i.e., Se), higher consumer tissue concentrations can be predicted not only as a function of thermodynamically driven energy transfer, they are also projected through stoichiometric relationships, i.e., chemical reactions forming strict ratios (Garvey & Whiles 2017). Elements accumulating at the autotrophic level move via the diet through the food web, with heterotrophs accumulating elements in their own stoichiometric ratios. In reality, biological systems do not strictly follow a conceptual model. Variation is introduced by rates of reproduction, consumption, and decomposition, as well as non-linear energy transfer introduced by detritivory, scavenging, and omnivory, not to mention differing environmental conditions (Huryn 1996). For this reason, the classic pyramid shape may be altered within any biological system, potentially contributing to strained trophic levels and ensuing cascade effects. Regardless of pyramid shape, trophic interactions are largely responsible for the flux of diet-driven

elemental components distributed within the organic fraction of an ecosystem.

Knowledge gaps currently exist relating to the influence of the primary producer trophic level on the bioaccumulation and trophic transfer of Se in aquatic ecosystems, particularly in freshwater environments. Selenium is a known essential micronutrient for most orders of life, including algae (Doucette et al. 1987). Its relative uptake kinetics are determined by the Se chemical species; SeIV shows no evidence for carrier mediated uptake, while SeVI and SeMet uptake show saturation kinetics, implicating carrier mediated processes that follow Michaelis-Menton kinetics (Fournier et al. 2006). As a result, SeIV enrichment has been found to be a linear function of ambient concentration, while SeVI and SeMet enrichment cannot be predicted by the same function of ambient Se concentration (Baines & Fisher 2001). More than solely a reflection of Se chemical species, enrichment into the primary producer varies based on the exposed algal species, one such example showing variance in SeIV enrichment of 4-5 orders of magnitude between tested algal species (Baines & Fisher 2001). Selenium enrichment and trophic transfer in the environment, however, is not a consequence of any single algal species, but rather a collection of participating taxa. Periphytic biofilms represent an important constituent of naturally-occurring aquatic primary producers. They are defined as assemblages of microorganismal communities, comprised of algae, bacteria, fungi, and/or detritus, that are associated with submerged substrates (Stevenson 1996), and can greatly vary in composition as a result of existing environmental factors (Lowe 1996). These assemblages represent a significant food source for aquatic invertebrates and have the potential to be a source of variability in the enrichment of Se and its trophic transfer to higher trophic levels. Research is beginning to emerge regarding the influence of periphytic community composition on the enrichment of Se, where uptake by different communities varies as a function of environmental conditions, community structure, and Se chemical species (Conley

et al. 2009; Friesen et al. 2017; Markwart et al. 2019).

Dynamic multi-pathway bioaccumulation models have been suggested as the best conceptual models to predict and understand metal and metalloid bioaccumulation. These models consider net bioaccumulation to be a factor of uptake rate from the diet, uptake rate from dissolved forms, and loss rates (Luoma & Rainbow 2005). Predictive modeling has produced a 1:1 relationship between measured Se body burdens in fish to predicted residues based on available food Se concentrations (Presser & Luoma 2009), suggesting the potential for an accurate prediction of Se uptake into higher consumer trophic levels based on dietary intake. This model has been less successful for the primary consumer trophic level, where dietary uptake remains the greatest exposure route, though not to the same extent. Aqueous uptake is believed to represent an important secondary Se exposure pathway for aquatic invertebrates, with variability in enrichment again showing inter-specific differences (Presser & Luoma 2009). Significant Se uptake through aqueous exposure has been reported for SeIV and SeMet in the invertebrate *Chironomus dilutus*, though probable Se uptake by dietary components within the exposure environment was not quantified (Franz et al. 2011).

In contrast to orders of magnitude differences in bioconcentration measured among taxa in the primary producer trophic level, trophic transfer factors (TTF) of Se from the primary consumer into the secondary consumer occur within a narrow range. In controlled laboratory studies, despite differences in prey, calculated TTFs for fish ranged between 0.5 – 2.8 when fed invertebrate diets (Baines et al. 2002; Ni et al. 2005; Matthews & Fisher 2008), and 0.5 – 1.3 when fed a piscivorous diet (Matthews & Fisher 2008). Field-derived measurements of Se trophic transfer are more complicated in comparison to laboratory-based values. A factor contributing to this difficulty includes complexity within natural food webs, where the composition of the diets of consumers is

inherently variable, and food web pathways are often uncertain or even unknown (Stewart et al. 2010). Crude or pooled sampling methods may contribute to obscured conclusions drawn from field-derived occurrences of elevated Se exposures. However, despite these uncertainties, TTFs measured for field-sampled fish species feeding on invertebrate prey agreed with laboratory-derived values, showing a range of 0.5 – 1.8 (Presser & Luoma 2009), though apparent agreement among test methods does not satisfy questions regarding trophic transfer efficiencies, a highly disputable factor that deserves further investigation.

1.5 Existing ecological selenium contamination research

1.5.1 Warm water contamination by selenium

Since adverse population effects were observed through Se contamination in well characterized cases at Belews Lake and Hyco Lake, North Carolina, USA, much research has been focused on comprehending the chemodynamics associated with the element and predicting threshold values for the protection of the environment. In these two instances, the lakes were impoundments acting as cooling reservoirs for a nearby coal-burning power plant, and therefore, received Se-laden effluents as a result. Highly concentrated wastewater was introduced into Belews Lake at concentrations measured between 100 – 200 $\mu\text{g Se L}^{-1}$ for a number of years, producing lakewide aqueous Se concentrations averaging 10 $\mu\text{g Se L}^{-1}$ at its height, and average sediment concentrations ranging from 4 – 30 $\text{mg Se kg}^{-1} \text{ dw}$ (Cutter 1991). Hyco Lake saw water Se concentrations as high as 7 – 14 $\mu\text{g Se L}^{-1}$ in areas nearest to the effluent source and sediment concentrations ranging between 3.6 – 35 $\text{mg Se kg}^{-1} \text{ dw}$ depending on distance to the effluent source (Cutter 1991). In both instances, SeIV was the primary chemical species occurring as waste from the combustion of coal. As a result of the Se contamination, both lakes incurred population shifts in their resident aquatic populations, and widespread reproductive failure of fish was

documented at Belews Lake (Cumbie & Van Horn 1978), while fish community declines occurred at Hyco Lake (Crutchfield 2000). In both instances, fish populations became dominated by fewer, more Se-tolerant species, with green sunfish (*Lepomis cyanellus*) emerging as one of the tolerant species in both instances (Barwick & Harrell 1997; Crutchfield 2000).

A great deal of attention has also been given to the fate of Se in the San Joaquin Valley and San Francisco Bay-Delta Estuary, both in the same region of central California, USA, where extensive agricultural development and oil refining has resulted in elevated Se transported through irrigation and drainage practices. The Kesterson Reservoir in the San Joaquin Valley became a series of terminal flow evaporative ponds, collecting irrigation drainwater with high levels of mineral salts to alleviate salinization of croplands, incurring the function that had been planned for the San Luis Drain (SLD) (Presser & Ohlendorf 1987). As a result of Se contamination, the Kesterson Reservoir saw a local extirpation of fish species, along with reproductive impairment in aquatic bird populations, observed as deformities and mortality in embryos and hatchlings (Ohlendorf 1989). Recognition of adverse effects of the failed containment project stemmed the end of agricultural irrigation inputs into the SLD and Kesterson Reservoir, and remediation measures were established. The San Francisco Bay-Delta Estuary also received Se-laden waters from irrigation discharge from the San Joaquin Valley, as well as effluents from oil refineries in the area and inflows from the Sacramento River (Presser & Luoma 2006). Selenium contamination in the San Francisco Bay-Delta Estuary were measured at levels that were sufficient to threaten reproduction in local aquatic species (Presser & Luoma 2006), and human health advisories were even posted based on tissue Se concentrations measured in diving ducks. Management and regulation of industrial inputs in these areas have been effective in reducing Se loading; however, the San Joaquin Valley and San Francisco Bay-Delta Estuary continue to be monitored for

persisting adverse impacts on local populations (Presser & Luoma 2013; David et al. 2015; Gundersen et al. 2017).

1.5.2 Canadian selenium contamination events

Canada has also seen problematic influxes of Se into the environment, particularly arising from mining operations. Given the differences in climate in northern environments, concern arose around potential disparities in the chemodynamics of Se in comparison to the greater researched, warmer ecosystems. The Elk Valley in British Columbia, Canada, has high-grade coal deposits, and consequently the mine disturbance has resulted in weathering and release of Se associated with pyrite (FeS_2). Effluent released from the five mines in the area can exceed $300 \mu\text{g Se L}^{-1}$, and the Elk River had concentrations that ranged from $5.8 - 9.6 \mu\text{g Se L}^{-1}$ at a monitoring site 60 km downstream of these discharges (Martin et al. 2008). In Elk Valley lentic habitats, biotic Se residues ranged from $3.9 - 12.3 \text{ mg Se kg}^{-1} \text{ dw}$ in benthic invertebrates and up to $76 \text{ mg Se kg}^{-1} \text{ dw}$ in fish, whereas local lotic habitats ranged from $2.7 - 9.6 \text{ mg Se kg}^{-1} \text{ dw}$ and $4 - 15 \text{ mg Se kg}^{-1} \text{ dw}$ in comparable invertebrate and fish tissues respectively (Minnow Environmental 2007). Consequently, local waterfowl have shown reduced hatchability (Harding et al. 2005), amphibian studies have shown a correlation between embryo Se residues and the prominence of deformities (Elk Valley Selenium Task Force 2008), and reproductive failure was observed in trout at embryo Se residues above $35 \text{ mg Se kg}^{-1} \text{ dw}$ (Rudolph et al. 2008; Nautilus Environmental & Interior Reforestation 2009). It should be noted that adverse reproductive effects were not statistically significant in the American dipper (Harding et al. 2005), and an initial attempt at establishing a site-specific Se effects threshold for cutthroat trout found no adverse effects on the fish offspring at concentrations up to $80 \text{ mg kg}^{-1} \text{ dw}$ embryo Se (Kennedy et al. 2000), though this research has been critiqued due to high reference site offspring mortalities and the absence of critical fecundity

data (Hamilton & Palace 2001), and subsequent attempts established an EC_{10} at $19 - 22 \text{ mg Se kg}^{-1} \text{ dw}$ (Rudolph et al. 2008; Nautilus Environmental & Interior Reforestation 2009). Related in part to data collected from a diversity of ecosystems within the Elk Valley Region, there are now site-specific water quality screening guidelines that account for differences between lotic and lentic habitats, where flowing lotic environments allow a greater threshold of Se within the systems without adverse consequences than waters of standing lentic environments (DeForest et al. 2017).

Much attention has also been focused on Se contamination in northern Saskatchewan, Canada, downstream of uranium mining and milling at Key Lake Operations. Treated effluent has been discharged into the David Creek drainage for over 30 years, through release into Wolf Lake and flow through a series of oligotrophic lakes, eventually draining into the Wheeler River. Discharge into this connected system has created a decreasing gradient in major ions and trace elements measured in surface waters and sediments, including Se (Klaverkamp et al. 2002; Muscatello et al. 2008). In 2004, regulators requested that Se in the treated effluent be decreased due to potential risk to fish populations, leading to a reduction in annual loading from 125 kg in 1998 to 16.6 kg in 2012 (Janz et al. 2014). Research has been ongoing in the region, leading to a compilation of data that has allowed for a comprehensive assessment of biogeochemical cycling and trophic dynamics of Se species in the cold, freshwater environment (Janz et al. 2014). Several studies describing Se content measured within numerous compartments (i.e., water, sediment, and biota) (Muscatello et al. 2008), as well as investigations using semicontrolled mesocosm and *in situ* caging studies (Driessnack et al. 2011; Phibbs et al. 2011; Franz et al. 2013) have contributed to a thorough understanding of this ecosystem. Periphyton scraped from mesocosm walls after 21 days accumulated Se to the range of $0.48 - 0.55 \text{ } \mu\text{g Se g}^{-1} \text{ ww}$, which was significantly greater than reference treatments measured at $0.03 \text{ } \mu\text{g Se g}^{-1} \text{ ww}$ (Driessnack et al. 2011). An *in situ* caging

study found differences in total Se concentrations among benthic invertebrate feeding groups, ranging from 6 – 25 $\mu\text{g Se g}^{-1}$ dw at Unknown Lake, and 3 – 13 $\mu\text{g Se g}^{-1}$ dw at the further downstream Delta Lake. Whole-body Se concentrations in caged and wild fish from the same experiment ranged from 2 – 20 $\mu\text{g Se g}^{-1}$ dw (Muscatello et al. 2008). Lake chub caged in Unknown Lake also showed a positive relationship between sediment total organic carbon and whole-body Se accumulation after 21 days (Phibbs et al. 2011). Of particular interest, a consistent finding from research at the Key Lake Operation was the domination of SeCys-like species within reference biota shifted to a concentration-dependent dominance by SeMet-like species in elevated exposure scenarios (Phibbs et al. 2011; Franz et al. 2011). The conclusion that excess Se accumulates as SeMet is especially important because it incorporates non-discriminately into peptides, where coding enzymes do not discriminate between SeMet and its sulphur analog, methionine (Moroder 2005). This renders SeMet the Se species of most concern due to its propensity to bioaccumulate and cause toxicity in aquatic food webs (Fan et al. 2002).

1.6 Model test organisms

1.6.1 Primary producers: *Stichococcus bacillarus* and natural biofilms

Selenium enrichment varies as a function of primary producer species (Riedel et al. 1991; Baines & Fisher 2001), so further research is necessary to elucidate the effect of this variability on Se transfer to higher trophic levels. As such, an objective of the present research was to characterize the bioaccumulation and trophic transfer of Se along a representative Canadian freshwater food chain under controlled conditions, with particular attention focused on the influence of the primary producer bioconcentration step. This was accomplished through analysis of inorganic, waterborne Se uptake by a monoculture of green alga, *Stichococcus bacillarus*, and secondarily through collection of and controlled Se exposure to a range of natural periphytic

biofilm communities collected from uncontaminated lakes, characterized by a range of limnological characteristics. The green alga *S. bacillarus* was chosen as the laboratory-grown monoculture species to assess Se enrichment and dietary trophic transfer because it belongs to a genus that possesses a wide ecological distribution, existing in a range of aquatic habitats, and was specifically selected based on its display of periphytic adhesion. Lakes chosen for periphytic biofilm collection had a variety of limnological characteristics, based on the assumption that biofilm community composition varies based on the environmental conditions. The enrichment of Se from the aqueous phase to the different biofilms and alga, and the further trophic transfer to a representative aquatic primary consumer, were analyzed and compared. The invertebrate species was further exposed to the laboratory algae monoculture with concurrent aqueous Se exposure and collected for use as the diet for the small bodied secondary consumer fish species.

1.6.2 Primary consumer: *Hyaletta azteca*

Primary consumers are considered a fundamental dietary source of Se to higher trophic levels, due to the combination of their potential to accumulate high Se body burdens, and their trophic position as the link between the Se bioconcentrating primary producer level, and higher, more sensitive consumer levels (Lemly 1993). Dietary trophic transfer of Se has been reported as highly variable among primary consumer taxa, hypothesized to be driven by physiological differences in food preference, assimilation efficiencies, and the rate of Se loss (Schlekat et al. 2002). Dynamic, multi-pathway bioaccumulation models have been suggested as the best conceptual models to explain metal and metalloid bioaccumulation (Luoma & Rainbow 2005). Data considering Se uptake pathways as they relate to food web structure are important for an accurate prediction of biokinetic Se dynamics among trophic levels.

In the present research, *H. azteca* was chosen as the aquatic invertebrate test species to

assess Se trophic transfer and bioaccumulation at the primary consumer level. This crustacean species is widely distributed in freshwaters in North and South America (Pennak 1989), and is considered one of the most sensitive freshwater species to contaminants (Phipps et al 1995). It has been commonly used in laboratory tests because it is relatively easy to culture, possesses a short maturation period and life cycle, and has established standardized experimental protocols (ECCC 2013). This amphipod species has been often used in ecotoxicological assessments, including toxicity hazard evaluations for Se (Halter 1980; Brasher & Ogle 1993; Brix et al. 2001), which provided ample data for reference to the current experimental design. Measured acute and chronic toxicities of waterborne Se have been found to vary, with LC₅₀ values ranging between 0.070 – 4.31 mg Se L⁻¹, due to differences in Se speciation, test methods, and variable water chemistries among experimental conditions (Pieterek & Pietrock 2012). The present subset of amphipods was acquired from an in-house culture at the Toxicology Centre, University of Saskatchewan, Saskatoon, SK, Canada. Selenium toxicity tests for this particular culture have been previously reported, showing a higher sensitivity to SeVI than a subsample of field-collected amphipods of the same species, though strain differences were likely a factor (Pieterek & Pietrock 2012). This finding corroborated another *in situ* study, where the laboratory-reared *H. azteca* were caged in effluent receiving waters downstream of the Key Lake Mining Operation (Robertson & Liber 2007). The purpose of the present study, however, was not an assessment of Se toxicity to the invertebrate, but rather a characterization of trophic transfer of the element.

1.6.3 Secondary consumer: *Pimephales promelas*

Higher oviparous consumer species have rightfully received the greatest amount of attention regarding impacts of Se exposure. A combination of plainly visible adverse effects, and the economic and social importance of the most impacted oviparous taxa has led to a succession

of investigations of the biodynamics and toxicity of Se in these taxa. A great deal of research has concentrated on using controlled laboratory conditions to further understand species-specific sensitivities, cellular modes of toxic action, and interacting factors in Se exposures and uptake (Palace et al. 2004; Holm et al. 2005; Eagles-Smith et al. 2009). Despite agreement regarding the teratogenic effects caused by Se, in large part resulting from maternal transfer and microinjection of the element into embryos representing a depuration strategy by the mother, knowledge gaps continue to persist regarding effects on fish reproduction. Information on trophic transfer of Se into fish has principally been derived from ecological-based assessments, where food web structure has often lacked characterization, highlighting questions regarding the true trophic transfer efficiency of the element (Stewart et al. 2010). For this reason, a significant focus of the present research was to evaluate the trophic and maternal transfer of Se into an oviparous secondary consumer species from dietary exposure, to gain fundamental insight regarding bioaccumulation within a defined, naturally-derived food chain.

As of late, a great deal of Se research has been compiled, related to both field- and laboratory-derived exposures, particularly in regard to its effects on fish. Many studies have characterized the impacts of elevated Se in field scenarios as it relates to fish and their surrounding conditions (Holm et al. 2005; Driessnack et al. 2011; Phibbs et al. 2011; Janz et al. 2014). Sublethal impacts of elevated exposure to Se on fishes have also been investigated, relating to cardiac and metabolic effects, swimming performance, and energy homeostasis through dietary exposures (McPhee & Janz 2014; Pettem et al. 2017; 2018). A microinjection route of exposure as a potential surrogate for maternally-transferred Se has been used to answer questions regarding molecular mechanisms of Se toxicity (Thomas & Janz 2016; Lane et al. 2019). However, objections have been raised related to the realism of biodynamics and effects caused by exposure to artificially-

dosed organo-Se (Rigby et al. 2014).

Research focused on answering the many knowledge gaps that continue to dominate understanding of Se have included much attention to particular model fish species. In the present research, *Pimephales promelas* was chosen as the test fish species to assess Se trophic transfer and bioaccumulation at the level of the secondary consumer. The fathead minnow belongs to the Cyprinidae family, the largest freshwater fish family. The fathead minnow range extends over the majority of central North America, reaching as far south as northern Mexico (Scott & Crossman 1973). This fish species has been the subject of a great deal of scientific examination, including many Se studies. Specific attributes that make the fathead minnow a suitable species for scientific inquiry include its small body size, its well-known culture care and easily-controlled reproductive strategies, and its annotated genome (Burns et al. 2016; Saari et al. 2017). For many decades, *P. promelas* has been a subject of Se research due in part to its tolerance of the element, as exemplified by the fish's persistence during Se contamination at Belews Lake (Barwick & Harrell 1997). For these reasons, the fathead minnow was determined to be an appropriate candidate for further Se research regarding the trophic transfer of the element in a controlled laboratory food chain. An adult fathead minnow reproductive assay was designed following methods established by an existent foodborne Se assay (Ogle & Knight 1989) and OECD guideline (OECD 2012), with the aim of producing an exposure regimen that was directly comparable to a recently published assay where the food source (Chironomidae) was artificially dosed with SeMet to establish trophic transfer efficiencies and maternal transfer to the offspring (Lane et al. 2019). This provided a means of estimating the relative contribution of SeMet into the fish's tissues in comparison to a diet simulating a more ecologically relevant exposure scenario. A subsample of progeny was also reared for an assessment of deformities.

1.7 Research goals and objectives

1.7.1 Scope of work

Ecological examples of adverse effects caused by elevated Se exposures in wild populations have been the focus of significant scientific inquiry. However, the complexity of those ecosystems involved render potential competing interactions and confounding factors difficult to decipher. For this reason, support is needed from highly controlled experiments where parameters are consistent and well characterized to dissect underlying mechanisms. Criticisms in existing experimental designs and improvements in analytical techniques continue to propel the research forward and highlight gaps in our apparent ecotoxicological understanding of the element. For instance, despite the established critical influence of the primary producer trophic level on Se bioconcentration and trophic transfer, understanding of kinetic processes at the base of the food web, isolation of abiotic and biotic components of sediments and periphyton, and trophic transfer variability to higher organisms are contributing factors that require further characterization (Stewart et al. 2010). The purpose of the present research thesis was to help clarify existing knowledge gaps, with particular attention to ecologically-relevant exposure scenarios and dietary trophic transfer initiated from the primary producer trophic level.

Not unlike other essential elements, organisms must accumulate small amounts of Se from the environment to perform normal physiological processes; however, they must also manage intake to avoid toxicity. The ecological risk of Se bioaccumulation and toxicity in freshwater environments cannot be predicted based solely on water quality and the element's dissolved concentration in the aqueous phase. There is evidence that the concentration of Se taken up by primary producers at the base of the food chain is preserved and sometimes slightly biomagnified as it is passed on to higher consumer organisms (Luoma & Presser 2009). Ultimately, the trophic

transfer concept would lead to the hypothesis that site-specific differences, primarily in food web composition and structure, could cause significant variation in Se uptake and biomagnification. Models are therefore needed that incorporate knowledge about the uptake and biotransformation processes of Se at the base of the food chain. Consequently, the purpose of the present research project was to elucidate the influence of primary producers on the ultimate uptake and trophic transfer of Se species in aquatic food webs as a means of extrapolation for ecologically-relevant scenarios. By analyzing Se uptake in a variety of periphyton communities, the relative importance of primary producer community composition to higher trophic level exposure was assessed through dietary pathways.

1.7.2 Experimental objectives

The overall goal of this research was to examine the enrichment of inorganic Se chemical species into a laboratory-grown primary producer and field-collected biofilm communities, for a comparative assessment of its trophic transfer into higher consumer species, including an oviparous vertebrate, the group most sensitive to Se exposure. The intention of this research was to contribute to the existing understanding of Se biodynamics, particularly in cold, freshwater ecosystems, for the further development of appropriate management strategies for the protection of aquatic ecosystems.

Objective 1: Establish the influence of periphyton community composition at the base of the food web on uptake of Se species and its transfer to the primary consumer trophic level. Specific aims included the determination of:

- i. Enrichment functions (EF) of aqueous Se species (SeIV and SeVI) from the water into environmentally-sampled, site-specific periphyton communities;
- ii. TTFs of Se transferred from these environmentally-sampled, site-specific periphyton communities to the laboratory invertebrate consumer, *H. azteca*.

Objective 2: Assess the respective uptake and trophic transfer of inorganic Se species (SeIV) through a representative cold, freshwater laboratory food chain. Specifically, this objective included the determination of:

- i. TTFs of Se to a laboratory aquatic invertebrate species, *H. azteca*, either exposed or unexposed to aqueous Se, and fed a selenized *S. bacillarus* diet;
- ii. TTFs of Se to a laboratory small-bodied fish species, *P. promelas*, fed a selenized *H. azteca* diet;
- iii. Analysis of effects caused by dietary Se exposure using a simulated food chain to other studies using SeMet-spiked food or embryo injection.

1.7.3 Null-hypotheses

H₀₁: The uptake rate and enrichment of inorganic Se species (SeIV and SeVI) from an aqueous exposure will not significantly differ among a laboratory grown, algal monoculture and different periphyton communities collected from various natural aquatic ecosystems;

H₀₂: Trophic transfer of Se species into the tissue of a representative primary consumer will not significantly differ between the use of selenized diets of laboratory grown, monoculture algae or periphyton communities collected from various natural aquatic ecosystems;

H₀₃: Bioaccumulation of Se species in a primary consumer will not significantly differ when exposure is through a selenized diet alone or in combination with an aqueous Se exposure;

H₀₄: Trophic and maternal transfer of Se will not significantly differ among secondary consumers fed different treatments of primary consumers varying in Se content;

H₀₅: Toxicity from dietary Se exposure to the secondary consumer species and their offspring will not significantly differ between the present simulated food chain and other studies using SeMet-spiked food or embryo injection in the same test species.

CHAPTER 2

SELENIUM OXYANION ENRICHMENT IN FIELD-COLLECTED BIOFILMS AND TROPHIC TRANSFER TO A PRIMARY CONSUMER

Preface

The research in this chapter was designed to assess the enrichment of inorganic selenium, as selenate or selenite, in field-collected biofilm sampled from diverse lentic waterbodies, and its trophic transfer to a primary consumer, *Hyalella azteca*. This chapter will be submitted to the journal Environmental Science and Technology. The anticipated citation is: Raes, K.A., Doig, L.E., Markwart, B., Liber, K., Janz, D.M., & Hecker, M. (2020). Selenium oxyanion enrichment in field-collected biofilms and trophic transfer to a primary consumer. *Environ Sci Technol*, (in preparation).

The author contribution to chapter 2 of this thesis were as follows:

Katherine Raes (University of Saskatchewan) designed the study, collected, processed, and analyzed all samples, performed all statistical analyses, and drafted the manuscript.

Lorne Doig (University of Saskatchewan) helped design the study, provided scientific input and guidance, reviewed and revised the manuscript, and provided comments and corrections.

Blue Markwart (University of Saskatchewan) helped design the study and provided scientific input.

Karsten Liber (University of Saskatchewan) helped design the study, provided scientific input and guidance, reviewed and revised the manuscript, and provided comments and corrections, and procured and provided funding required to conduct the research.

David Janz (University of Saskatchewan) helped design the study, provided scientific input and guidance, reviewed and revised the manuscript, and provided comments and corrections, and procured and provided funding required to conduct the research.

Markus Hecker (University of Saskatchewan) helped design the study, provided scientific input and guidance, reviewed and revised the manuscript, providing comments and corrections, and procured and provided funding required to conduct the research.

2.1 Abstract

Toxicological concern has been raised over the mobilization and accumulation of selenium (Se) in aquatic ecosystems, particularly for oviparous vertebrates where exposure to excess Se causes reproductive failure and teratogenic effects. Significant uncertainties exist relating to dietary Se trophic transfer to higher organisms, originating from bioconcentration and biotransformation at the base of the food web. The present study quantified the trophic transfer of Se to a primary consumer from diets of natural, field-collected biofilms that were exposed to Se oxyanions in the laboratory (control [$0.33 \mu\text{g Se L}^{-1}$], selenite [SeIV] and selenate [SeVI], each at concentrations of 5 and $25 \mu\text{g Se L}^{-1}$). The amphipod *Hyaella azteca*, a primary consumer characteristic of Canadian freshwater ecosystems, grazed on the Se-exposed biofilm communities to determine trophic transfer efficiencies as a function of algal community structure. SeIV was transferred following a concentration-dependent relationship, and final biofilm and amphipod tissue Se concentrations varied by site. Final mean biofilm tissue Se concentrations ranged between 6.3 to $15 \mu\text{g Se g}^{-1} \text{ dw}$ in the low SeIV treatment and 17 to $45 \mu\text{g Se g}^{-1} \text{ dw}$ in the high SeIV treatment, compared to a range between 1.4 to $2.1 \mu\text{g Se g}^{-1} \text{ dw}$ in the controls. Final mean amphipod tissue Se concentrations ranged between 2.7 to $6.5 \mu\text{g Se g}^{-1} \text{ dw}$ in the low SeIV treatment and 5.8 to $18 \mu\text{g Se g}^{-1} \text{ dw}$ in the high SeIV treatment, compared to a range between 0.7 to $2.8 \mu\text{g Se g}^{-1} \text{ dw}$ in the controls. Trophic transfer factors from the biofilm to the invertebrate varied, ranging from 0.15 to 0.51 in both the low and high SeIV treatments. Selenium trophic transfer was likely influenced by multiple factors including Se species, biofilm biomass, and the proportion of cyanobacteria. Uptake of SeVI into the amphipods was not significantly different across treatments compared to the controls. This research will help to improve environmental risk assessment strategies in the management of Se in aquatic environments.

2.2 Introduction

Selenium (Se) is a naturally occurring element, whose global distribution is linked to geologic formations of organic-rich depositional marine basins (Presser et al. 2004a). Though natural cycling contributes to fluxes in global Se distribution, environmental contamination occurs primarily as a by-product of anthropogenic activities (Presser et al. 1990), including mining activities, fossil fuel combustion, oil refining, fertilizer production, and agricultural irrigation (Cumbie and Van Horn 1978; Cutter 1991; Muscatello and Janz 2009). Predominantly inorganic Se is released by these activities, in the form of selenite (+4 oxidation state) or selenate (+6 oxidation state) oxyanions, depending on the source or processing of the Se-laden materials (Maher et al. 2010). Selenium is further mobilized and redistributed to aquatic ecosystems via effluents, or through the contact of Se-containing matrices with water (Young et al. 2010). In aquatic ecosystems, dissolved Se is efficiently bioaccumulated and biotransformed by aquatic microorganisms into organo-Se compounds, which are readily transferred to higher trophic levels primarily through dietary pathways (Stewart et al. 2004; Janz et al. 2014). Despite the element's essentiality among organisms, Se contamination in the aquatic environment can result in major ecosystem disruption following an ensuing cascade of trophic transfer events (Lemly 1985).

The ecological risk of Se bioaccumulation and toxicity in freshwater environments has proven difficult to predict based solely on aqueous Se concentration and water quality. Not unlike other essential but potentially toxic elements, organisms must uptake Se from the environment to perform normal physiological processes (Gatlin and Wilson 1984; Hilton et al. 1980), while also managing intake to avoid toxicity (Lemly 1993). The greatest bioconcentration step of Se from the aqueous phase occurs at the level of the primary producer, and the largest source of Se for most higher aquatic organisms is from the diet (Stewart et al. 2010). For this reason, a comprehensive

understanding of the extent of Se accumulation in aquatic food webs begins at the primary producer base. High-affinity Se uptake through specific cellular pathways, facilitated by the essentiality of Se, has proven efficient even in the presence of low ambient and dietary Se concentrations, to accumulate sufficient Se concentrations for normal physiological functions (Riedel et al. 1991; Fournier et al. 2006). Uptake of Se into freshwater algae tissues is governed in part by chemical speciation. For example, SeVI and selenomethionine (SeMet) follow saturation kinetics due to uptake by specific transmembrane carrier proteins, while SeIV uptake occurs predominantly by passive absorption, kinetically shown as a linear function of ambient concentration (Fournier et al. 2006). Bioaccumulation of Se is therefore expected to vary in part as a function of species-specific uptake rates, related to the presence of cellular uptake pathways, among other factors such as chemical speciation and concentration, and the water chemistry of the surrounding environment; however, the relative influence of these factors remains elusive (Bowie et al. 1996).

Diet-driven trophic transfer of Se points to a critical influence of primary consumers in linking Se-enriched primary producers to Se tissue levels in higher consumer organisms. The concentration of Se taken up by primary producers at the base of the food chain is preserved and, in some circumstances, biomagnified as it is passed on to higher consumer organisms (Luoma and Presser 2009). This relationship becomes more variable at higher tissue Se concentrations, when factors such as feeding inhibition become more influential in the ultimate uptake by organisms (Croteau and Luoma 2008). Ultimately, site-specific differences in food web composition and structure would lead to significant variation in Se uptake and biomagnification. This specificity in environmental differences makes overarching regulations among jurisdictions difficult to apply.

Models are therefore needed to integrate uptake and biotransformation processes of Se at the base of the food chain relative to site-specific environmental factors.

The purpose of this study was to elucidate impacts of Se uptake at the level of the primary producer on the uptake and trophic transfer of Se in an aquatic food web. Specifically, this study characterized enrichment and trophic transfer of Se by: 1) a laboratory-grown, monoculture green algae Se exposure, followed by dietary Se exposure to an amphipod alone or in combination with an aqueous Se exposure, as a function of time; and 2) a field-grown, laboratory-based biofilm Se exposure, using natural biofilm communities collected from uncontaminated lakes that were characterized by a range of limnological properties, and subsequently exposed to experimental Se conditions and fed to an amphipod. The ultimate purpose of this research was to characterize Se uptake and trophic transfer into an ecologically-relevant food chain, for extrapolation and comparison to ecological scenarios.

2.3 Materials and methods

2.3.1 Enrichment and trophic transfer of selenite from *S. bacillarus* to *H. azteca*

Stichococcus bacillarus was originally acquired from the Canadian Phycological Culture Centre (CPCC) at the University of Waterloo, Waterloo, ON, Canada and maintained in-house at the Aquatic Toxicology Research Facility (ATRF) at the Toxicology Centre (University of Saskatchewan, SK, Canada). The alga was maintained following the Environment and Climate Change Canada Biological Test Method for Growth Inhibition in Freshwater Alga (ECCC 2007). The test culture was maintained in Bold's Basal Medium for green algae (Stein et al. 1973) at an ambient temperature of 24°C under 16:8 hours light:dark photoperiod in a controlled environmental incubator. Evenly distributed cell concentrations of *S. bacillarus* were exposed to aqueous selenite (SeIV; Na₂SeO₃) at concentrations of 0 (control), 5, and 25 µg Se L⁻¹ for eight

days. Aqueous exposures were refreshed every two days, replacing a third of the dosed growth medium. On the eighth day of exposure, algae were separated from the aqueous exposure by centrifugation at 1800 g for 10 min, with a mean mass of 0.61 ± 0.04 g ww per 50-mL vessel, and stored at 4°C in a refrigerator. Subsamples of dosed algae were taken on days 0 and 8 for analysis of total Se concentrations. Algae were resuspended in *H. azteca* exposure water at the respective aqueous SeIV concentrations (0.3 [control], 5, and 25 $\mu\text{g Se L}^{-1}$) for feeding to the amphipods.

The amphipod *H. azteca*, was acquired from an in-house culture at the Toxicology Centre. *Hyalella azteca* were maintained following guidance from the Environment and Climate Change Canada *Hyalella azteca* Biological Test Method for Survival and Growth in Sediment and Water (ECCC 2013). The test culture was maintained in dechlorinated municipal water with bromine supplementation at an ambient temperature of 24°C under a 16:8 hours light:dark photoperiod in a controlled environmental chamber. Ten subadult *H. azteca* (7-10 days old) were added to 300-mL beakers with 150 mL of water and 100 g of silica sand substrate. To determine the relative influence of aqueous Se on uptake into the amphipod, distinct treatments were used where no aqueous Se was added to the amphipod test vessels (Aq_{Con}) or an aqueous Se exposure was added (Aq_{Se}) at concentrations corresponding to the respective algae treatments (0.3 [control], 5, and 25 $\mu\text{g Se L}^{-1}$). In order to maintain intended aqueous Se conditions among treatments and minimize Se loss from the exposed algae, *H. azteca* were fed 1 mL of dosed algae every two days in all treatments, allowed to graze for 24 hours, then test vessels were acid washed and refreshed to their respective aqueous Se concentrations. Amphipods were collected for analysis of total Se concentrations on days 0, 1, 7, and 15.

2.3.2 Enrichment and trophic transfer of selenium from field-grown biofilms to *H. azteca*

2.3.2.1 Site selection

Field sites for biofilm collection corresponded to those reported in Markwart et al. (2019). Briefly, site selection involved measuring various water chemistry parameters (dissolved oxygen (DO), temperature, hardness, alkalinity, conductivity, pH) of fifteen lakes and ponds located in the Boreal Plains of Northern Saskatchewan, Canada, in May 2016. Four water bodies used for biofilm collection in the present study were selected based on variation in aqueous chemistry characteristics, following the principle that biofilm community composition should vary based on species-specific differences among environmental conditions (Falkowski et al. 1998; Roelke et al. 2013; Lee et al. 2015). The following were the selected field sites with accompanying GPS coordinates: Chris' Pond (54°17'7.50"N, 104°40'21.36"W); Summit Lake (54°9'49.80"N, 104°45'43.14"W); Roadside Lake (54°17'2.40"N, 104°38'30.66"W); Smeaton Pond (53°44'26.82"N, 104°35'38.64"W) (Fig 2.1). General water quality and light characteristics measured at each of the four field sites at the time of initial sampler deployment (July 2016) and respective final sampler collection (September / October 2016) are summarized in Table 2.1.

2.3.2.2 Biofilm collection

Biofilm samplers were designed and constructed at the Toxicology Centre, following the same design as Markwart et al. (2019). Briefly, samplers were each composed of five abraded soda-lime-silicate glass tiles (20 cm x 20 cm x 5 mm) serving as substrate for biofilm colonization, supported by a PVC pipe frame. Five samplers were situated in the littoral zone of each of the four uncontaminated water bodies, for a total of 25 glass sampling tiles per site. Samplers were retrieved after two months and transported in coolers filled with site water to the Toxicology Centre. The biofilm from each site was tested individually, with respective samplers being collected every

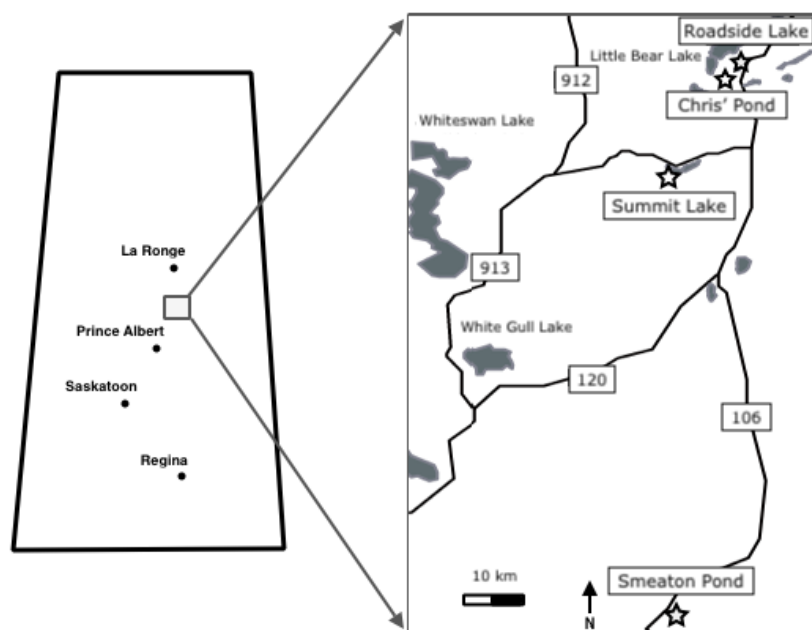


Figure 2.1. Locations of selected sampling sites for field-grown biofilm collection in northern Saskatchewan, Canada. Sampling sites were Smeaton Pond, Summit Lake, Chris' Pond, and Roadside Lake.

Table 2.1. Summary of general water quality parameters, photosynthetically active light (PAR), and aqueous Se concentrations of the four sampled waterbodies. The top row represents measurements taken at the time of initial sampler deployment (July 2016), and the bottom row represents measurements taken at the time of sampler collection (Sept/Oct 2016) for each respective field site.

Field Site	Temperature (°C)	DO (mg L⁻¹)	Conductivity (μS cm⁻¹)	pH	Alkalinity (mg CaCO₃ L⁻¹)	Hardness (mg CaCO₃ L⁻¹)	PAR (μmol m⁻² s⁻¹)	Se (μg L⁻¹)
Chris' Pond	23	8.4	308	7.9	162	154	243	0.07
	12	8.5	285	8.1	140	142	198	0.07
Summit Lake	22	9.6	285	8.3	160	142	758	0.04
	13	11	278	8.2	148	134	581	0.01
Roadside Lake	22	8.7	94	7.7	36	42	119	0.08
	13	8.7	120	7.9	40	60	83	0.06
Smeaton Pond	18	3.9	789	7.5	204	228	551	0.10
	7.6	3.3	776	7.3	180	200	56	0.05

subsequent week for four weeks.

2.3.2.3 Experimental setup

All exposures were conducted in the ATRF. Ambient temperature was controlled to $18 \pm 1^\circ\text{C}$ with a 16:8 hours light:dark cycle. All glassware and plasticware used for experimentation were disinfected with 5% sodium hypochlorite solution (Clorox, Oakland, CA, USA), acid-washed with 1M hydrochloric acid (Certified ACS Plus, Fisher Scientific, Hampton, NH, USA), and rinsed with ultrapure water ($17\text{ M}\Omega\text{-cm}$; Barnstead, Thermo Scientific, Waltham, MA, USA). For the initial eight-day pre-exposure period (day -7 to day 0), with the purpose of allowing the biofilms to reach Se steady state (i.e., no statistical difference in measured Se content between subsequent sampling periods) prior to amphipod addition, samplers were placed in 20-L aquaria with a translucent cover, containing aeration, $0.2\text{ }\mu\text{m}$ filtered dechlorinated municipal water and respective aqueous Se concentrations. Nominal treatment concentrations for the exposure period included five replicates ($n=5$) each for: a control (background: $0.33\text{ }\mu\text{g Se L}^{-1}$), and two concentrations ($5\text{ }\mu\text{g Se L}^{-1}$ and $25\text{ }\mu\text{g Se L}^{-1}$) of each selenite and selenate. Moving forward, treatments will be referred to as either ‘low’ or ‘high’ in regard to Se nominal concentrations (5 and $25\text{ }\mu\text{g Se L}^{-1}$, respectively) and the tested compound by its oxidation state (SeIV for selenite and SeVI for selenate) (e.g. low SeVI refers to the nominal $5\text{ }\mu\text{g Se L}^{-1}$ selenate treatment). Aquaria received 75% water replacements every second day to renew aqueous Se exposures. The amphipod exposure commenced after the eight-day pre-exposure period (day 0). Sampler glass tiles were divided into individual 4-L polypropylene exposure containers with translucent covers, continuous aeration, and aqueous Se exposures with the respective water treatments. Twenty laboratory cultured *H. azteca* sub-adults (7-10 days old) were added to each replicate, for a total of five replicates per experimental treatment. The amphipods were then allowed to graze on the biofilms

for 14 days with 50% water replacements every second day to maintain aqueous concentrations of Se.

2.3.2.4 Sampling design

Samples were collected for water quality analysis (DO, temperature, conductivity, pH, hardness, and alkalinity) from all aquaria on pre-exposure days -7 and -3, and from a subsample of exposure vessels (n=3) on days 0 and 14 for all tests, to ensure consistency throughout experimentation. Temperature and DO were measured with a portable meter (YSI Professional Plus Quatro, Xylem, Yellow Springs, OH, USA), conductivity and pH with bench top probes (Model 170 Conductivity Meter, ATI Orion, Boston, MA, USA; Routine Pro pH Electrode, Mettler Toledo, Langacher, Switzerland), hardness and alkalinity by titration (Total Hardness Test Kit & Alkalinity Test Kit, LaMotte, Chestertown, MD, USA). Photosynthetically active radiation (PAR) was also measured at the top and bottom of exposure vessels using a 2π quantum sensor (Model MQ-500, Apogee Instruments, Logan, UT, USA). Mean (\pm SE) water quality parameters and light measurements throughout the Se exposure period are summarized in the appendix (Tables A.2.1 & A.2.2).

Samples for dissolved Se analysis were also collected from all aquaria on pre-exposure days -7 and 0, as well as before and after water changes on day -3, and from a subsample of exposure vessels (n=3) from each treatment on day 14. Any differences in dissolved Se after test water renewals were captured through the sampling before and after water renewals, which were found to be negligible. Samples for dissolved Se analysis were collected in 8-mL HDPE sample bottles using syringe filters (0.45- μ m pore size, polyethersulfone membrane, VWR International, Radnor, PA, USA) and acidified to 5 % with high-purity nitric acid (HNO₃) (Optima Grade Nitric Acid, Fisher Scientific, Hampton, NH, USA). Mean (\pm SE) dissolved total Se concentrations

measured in exposure waters are summarized in the appendix (Tables A.2.1 & A.2.2). Mean dissolved Se concentrations were within 20% of nominal values for all treatments.

Biofilms were sampled from all replicates on days -7, 0, and 14 for Se tissue concentrations and community composition analyses. Biofilms were sampled by scraping a known area on the glass tiles using a ceramic blade. Glass tiles were scraped along the vertical axis to account for any potential spatial variability in the biofilm matrix. Biofilm samples for Se analysis were collected in centrifuge tubes (50-mL HDPE Centrifuge Tubes, VWR, Radnor, PA, USA), and resuspended in ultrapure water. Tubes were centrifuged at 1800 g for 10 min, the supernatant decanted and replaced with new ultrapure water, for a total of three rinses. Rinsed samples were flash frozen in liquid nitrogen and stored at -20°C until being freeze-dried. Freeze dried samples were weighed to determine mass/area (mg dw cm^{-2}) on sampling glass tiles and acid digested for Se tissue analysis.

Hyalella azteca were sampled for dry weight measurements and total Se tissue concentration analysis prior to exposure on day 0 (n=2), and surviving organisms were retrieved and counted at the end of the exposure period on day 14 (n=5). Organisms were placed in clean culture water for 10 min, transferred to a 1 mM ethylenediaminetetra-acetic acid (EDTA; Sigma Chemical Co., St. Louis, MO, USA) solution for 15 min to remove any Se adsorbed to the surface of the amphipods, and subsequently rinsed with ultrapure water. Amphipods were not gut purged, to represent a diet that would be encountered in the environment. Samples were flash frozen in liquid nitrogen and stored at -20°C until freeze dried. Invertebrate dry weights were measured after freeze drying, and then organisms were acid digested prior to analysis of total Se concentrations.

2.3.2.5 Analyses

All Se analyses were performed at the Toxicology Centre using inductively coupled plasma

mass spectrometry (8800 ICP-MS Triple Quad, Agilent Technologies, Santa Clara, CA, USA) with a mean minimum detection limit of $0.06 \pm 0.03 \mu\text{g Se L}^{-1}$. Dissolved Se concentrations were measured directly from filtered and acidified test waters. Biofilm and *H. azteca* Se concentrations were measured following digestions. High purity, 69% nitric acid (1 mL) and high purity, 30% hydrogen peroxide (0.67 mL) (Sigma Aldrich, St. Louis, MO, USA) were added to teflon digestion vessels with pre-weighed and dried sample materials. Capped vessels were placed in a MARS-5 microwave digestion system (EM Corporation, Matthews, NC, USA) and held at 160°C for 20 min. Digests were filtered and diluted to 2% HNO₃ before analysis. Certified reference material (TORT-3 lobster hepatopancreas reference material for trace metals, National Research Council Canada, Ottawa, ON, CA; 1640a trace elements in natural water, National Institute of Standards and Technology, Gaithersburg, MA, USA), duplicates, and method blanks were used for digestions and instrumental analytics to ensure analytical accuracy and validity. Enrichment factor (EF) and trophic transfer factor (TTF) were calculated (Eq. 2.1-2.2):

$$\text{EF} = \text{primary producer tissue concentration } (\mu\text{g Se g}^{-1}) / \text{aqueous concentration } (\text{mg Se L}^{-1}) \quad (2.1)$$

$$\text{TTF} = \text{predator tissue concentration } (\mu\text{g Se g}^{-1}) / \text{prey tissue concentration } (\mu\text{g Se g}^{-1}) \quad (2.2)$$

Biofilm samples for community composition analysis using light microscopy were collected using the scraping method described above. Samples were preserved in 1% glutaraldehyde and stored at 4°C. Microscopic identification and cell/colony counts followed the US-EPA Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers (1999). Algae from three replicates (n=3) per site were identified to the lowest taxonomic level possible using keys from Dillard (1999), Bellinger and Sigee (2010), and Agriculture and Agri-Food Canada's Algae Identification Lab Guide (2011). Counting units were measured with an ocular micrometer

to determine the average size of identified cells, and relative biovolumes were calculated using size measurements from equations in Hillebrand et al. (1999) and multiplied by cell counts.

2.3.3 Statistical analyses

For data relating to the trophic transfer of Se from *S. bacillarus* to *H. azteca*, a 1-way ANOVA and Dunnett's multiple comparison tests were employed to assess Se enrichment in the diets. A 2-way repeated measures ANOVA and Tukey tests for multiple comparisons were used to analyze Se uptake into the amphipods treatments within each sampling day ($\alpha=0.05$). For multiple comparisons, the Tukey correction was employed. Data from the natural biofilm Se exposure were noncompliant with parametric assumptions of normal distribution (Kolmogorov-Smirnov) and homogenous variance (Levene's test) despite transformations, and were therefore analyzed using non-parametric statistical tests. Mean uptake of Se into biofilms and *H. azteca* were analyzed by treatment for each field site, and mean biofilm biomass and mean *H. azteca* growth over the duration of the exposures were pooled for each field site ($\alpha=0.05$). These data were analyzed using Kruskal-Wallis tests followed by Dunn's tests for multiple comparisons.

2.4 Results

2.4.1 Enrichment and trophic transfer of selenite from *S. bacillarus* to *H. azteca*

Selenium accumulated in amphipods exposed to elevated dietary Se, with the highest invertebrate tissue residues occurring in treatments with concurrent waterborne SeIV (Fig 2.2). The green algae diet had concentrations of 2.4 ± 0.14 and 6.2 ± 0.48 $\mu\text{g Se g}^{-1}$ dw in the 5 and 25 $\mu\text{g L}^{-1}$ aqueous SeIV treatments, respectively, concentrations that were significantly elevated in comparison to the 0.08 ± 0.04 $\mu\text{g Se g}^{-1}$ dw in the control diet (ANOVA: $F_{2,6}=114$, $p<0.01$). A significant relationship was measured in amphipod Se concentrations based on the treatment

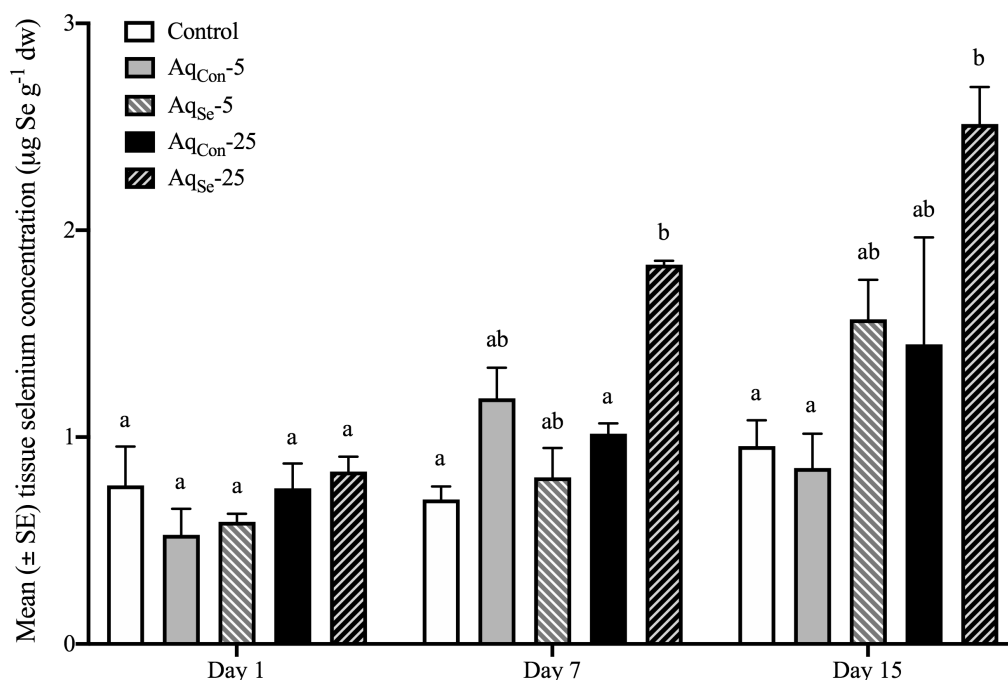


Figure 2.2. Mean (\pm SE) *H. azteca* Se concentrations ($\mu\text{g Se g}^{-1}$ dw) over three sampling periods (days 1, 7, and 15) with exposure to respective aqueous and dietary conditions. The five treatments were Control (white) [diet: $0.08 \mu\text{g Se g}^{-1}$ dw; aqueous: $0.3 \mu\text{g Se L}^{-1}$], AqCon-5 (grey) [diet: $2.4 \mu\text{g Se g}^{-1}$ dw; aqueous: $0.3 \mu\text{g Se L}^{-1}$], AqSe-5 (grey with lines) [diet: $2.4 \mu\text{g Se g}^{-1}$ dw; aqueous: $5 \mu\text{g Se L}^{-1}$], AqCon-25 (black) [diet: $6.2 \mu\text{g Se g}^{-1}$ dw; aqueous: $0.3 \mu\text{g Se L}^{-1}$], and AqSe-25 (black with lines) [diet: $6.2 \mu\text{g Se g}^{-1}$ dw; aqueous: $25 \mu\text{g Se L}^{-1}$]. Different letters (a-b) represent a significant difference ($p < 0.05$) in Se concentrations among treatment groups within respective sampling periods.

group and time of sampling (RM ANOVA: Treatment*Time – $F_{8,20}=3.2$, $p=0.02$). Aside from the Aq_{Con}-5 treatment, there was a trend of increasing tissue Se residues measured in the amphipods across all sampling days, suggesting a steady state may not have been reached in this exposure period. On the seventh day of exposure, concentrations in the Aq_{Se}-25 treatment group were significantly different from the controls and Aq_{Con}-25 treatment groups ($p<0.01$), showing a 2.6-fold increase relative to the controls.

At the termination of experimentation, both treatments with waterborne Se exposures showed increased uptake relative to respective treatments without waterborne Se exposures. The Aq_{Se}-5 treatment group showed a 1.8-fold increase compared to the Aq_{Con}-5 treatment group, and the Aq_{Se}-25 treatment group showed a 1.7-fold increase compared to the Aq_{Con}-25 treatment group. However, the only statistically significant differences were measured in the Aq_{Se}-25 treatment group relative to the control and Aq_{Con}-5 treatment groups ($p=0.01$). The Aq_{Se}-25 treatment showed a 2.6-fold increase relative to the controls. As a result, TTFs were lower in the treatment groups without concurrent aqueous Se exposure, calculated as 0.35 ± 0.07 and 0.66 ± 0.08 for the Aq_{Con}-5 and Aq_{Se}-5 treatments, and 0.24 ± 0.09 and 0.41 ± 0.03 in the Aq_{Con}-25 and Aq_{Se}-25 treatments, respectively, after 15 days of exposure.

2.4.2 Field-grown biofilm algal identification and enrichment of selenium

Dominant identified algal phyla generally varied by collection site (Table 2.2). Roadside Lake and Chris' Pond were both dominated by chlorophytes (91 ± 51 % and 74 ± 7.8 %, respectively), with a secondary presence of Bacillariophyta (7.4 ± 4.1 % and 22 ± 4.9 %, respectively). Summit Lake was largely dominated by bacillariophytes (55 ± 10 %), with chlorophytes ($22 \pm 9.3\%$) and cyanophytes (21 ± 10 %) composing secondary proportions. The assemblage collected from Smeaton Pond had the highest proportion of cyanophytes (38 ± 12 %),

Table 2.2. Summary of mean (\pm SE) proportion algal biovolume (%) of identified taxon in biofilms (n=3) collected from four field sites.

Taxonomic Classification			Proportion Algal Biovolume (%)			
Domain	Phylum	Genus	Chris' Pond	Summit Lake	Roadside Lake	Smeaton Pond
Eukaryota	Chlorophyta	<i>Asterococcus</i>		0.5 \pm 0.3		
		<i>Bulbochaete</i>	17.7 \pm 1.6	1.6 \pm 0.4	2.2 \pm 1.2	
		<i>Chaetopeltis</i>	0.8 \pm 0.4	2.8 \pm 2.8		
		<i>Chaetosphaeridium</i>			1.5 \pm 0.5	
		<i>Chaetophora</i>	3.5 \pm 0.6	4.9 \pm 2.5		
		<i>Chlorella</i>				0.4 \pm 0.3
		<i>Chlorococcum</i>			13.5 \pm 4.5	
		<i>Cladophora</i>			13.8 \pm 6.4	
		<i>Coleochaete</i>	51.9 \pm 5.2	7.5 \pm 1.1	35.9 \pm 19.7	
		<i>Dichotomosiphon</i>				20.6 \pm 20.6
		<i>Pediastrum</i>		0.5 \pm 0.1		
		<i>Scenedesmus</i>		0.1 \pm 0.1	0.1 \pm 0.1	
		<i>Spirogyra</i>			11.8 \pm 6.1	
		<i>Ulothrix</i>		2.7 \pm 1.0		
		<i>Zygnema</i>		1.8 \pm 1.0	12.4 \pm 12.4	
		Total	73.9 \pm 7.8	22.3 \pm 9.3	91.1 \pm 51.0	21.0 \pm 20.9
Eukaryota	Bacillariophyta	<i>Achnanthes</i>		0.9 \pm 0.2		
		<i>Amphora</i>	9.0 \pm 2.8			
		<i>Cocconeis</i>			0.8 \pm 0.8	
		<i>Cyclotella</i>		3.1 \pm 0.9		
		<i>Cymbella</i>		22.9 \pm 3.7	0.7 \pm 0.3	
		<i>Diatoma/Tabellaria</i>	0.4 \pm 0.1	1.6 \pm 0.6	0.9 \pm 0.4	
		<i>Eunotia</i>		0.3 \pm 0.1		
		<i>Fragillaria/Synedra</i>	1.6 \pm 0.1	5.6 \pm 0.4	0.7 \pm 0.4	0.5 \pm 0.3
		<i>Gomphonema</i>		1.4 \pm 0.2	1.3 \pm 0.6	
		<i>Navicula</i>		8.6 \pm 1.9	0.5 \pm 0.2	9.8 \pm 7.5
		<i>Peronia</i>				
		<i>Pinnularia</i>	11.1 \pm 2.0	7.4 \pm 1.2		29.5 \pm 10.0
		<i>Pleurosigma</i>		0.2 \pm 0.2		
		<i>Rhoicosphenia</i>		2.5 \pm 0.7	2.6 \pm 1.4	
		Total	22.1 \pm 4.9	54.5 \pm 10.1	7.4 \pm 4.1	39.8 \pm 17.8
Eukaryota	Ochrophyta	<i>Chrysamoeba</i>			0.5 \pm 0.5	
		<i>Chrysidiastrium</i>				1.1 \pm 1.1
		<i>Heribaudiella</i>				
		<i>Ochromonas</i>				0.2 \pm 0.2
		<i>Synura</i>		2.5 \pm 1.3		
Eukaryota	Rhodophyta	Total	0 \pm 0	2.5 \pm 1.3	0.5 \pm 0.5	1.3 \pm 1.3
		<i>Asterocytis</i>			0.7 \pm 0.5	
		<i>Audouinella</i>			0.2 \pm 0.2	
Prokaryota	Cyanophyta	Total	0 \pm 0	0 \pm 0	0.9 \pm 0.7	0 \pm 0
		<i>Aphanothece</i>	2.5 \pm 0.5	0.2 \pm 0.2	0.1 \pm 0.0	12.8 \pm 6.4
		<i>Chroococcus</i>	0.1 \pm 0.1			
		<i>Merismopedia</i>	0.1 \pm 0.1	0.1 \pm 0.1		
		<i>Microcystis</i>				3.1 \pm 1.5
		<i>Nostoc</i>				22.0 \pm 3.9
		<i>Oscillatoria</i>	1.3 \pm 0.7	20.4 \pm 9.7		
		Total	4.0 \pm 1.4	20.7 \pm 10.0	0.1 \pm 0	37.9 \pm 11.8

with a similar proportion of bacillariophytes ($40 \pm 18 \%$), and a lesser proportion of chlorophytes ($21 \pm 21 \%$).

Following differences observed in the algal community composition of field-collected biofilms, statistically significant differences were observed in biofilm biomass per unit area from the biofilms collected from respective sites, measured as the mean biomass across pre-exposure and exposure sampling periods for all treatments (K-W: $H=45$, $p<0.01$) (Fig 2.3). Summit Lake biofilms showed the greatest biomass per unit area for all sites ($p<0.01$), with a mean value of $1.7 \pm 0.15 \text{ mg dw cm}^{-2}$, 6.1-fold greater than the closest site's mean biofilm biomass, Chris' Pond, which measured $0.28 \pm 0.07 \text{ mg dw cm}^{-2}$. Roadside Lake and Smeaton Pond biofilms showed further reduced mean biofilm biomasses, with a mean of 0.04 ± 0.01 and $0.20 \pm 0.06 \text{ mg dw cm}^{-2}$, respectively.

When comparing the bioconcentration of Se among site-specific biofilms, consistent trends occurred. Across both treatment and site, Se uptake into the biofilms consistently showed concentration-dependent bioconcentration of SeIV, and comparably low uptake of SeVI. Site-specific analyses showed significant differences in relative SeIV uptake into biofilms between treatments at all sites (K-W: $H=23.5 - 30$, $p<0.01$), with consistent trends existing among treatments, where Se bioconcentration increased with increasing dissolved SeIV (Figure 2.4). Statistically significant concentration-dependent SeIV bioconcentration was measured in Chris' Pond and Smeaton Pond biofilms, where low SeIV tissue residues were greater than respective controls ($p<0.01$), reaching 11 ± 0.7 and $15 \pm 3.5 \text{ } \mu\text{g Se g}^{-1} \text{ dw}$, respectively. High SeIV tissue residues were further elevated ($p<0.01$), with Chris' Pond and Smeaton Pond biofilms averaging 41 ± 5.7 and $45 \pm 4.5 \text{ } \mu\text{g Se g}^{-1} \text{ dw}$, respectively. Summit Lake biofilms assimilated the least SeIV, a finding that was particularly apparent in the high treatment where mean tissue Se residues

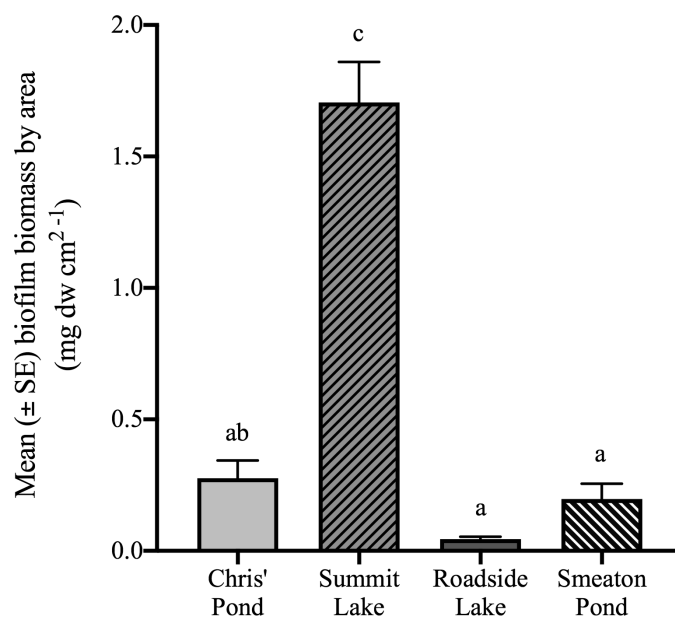


Figure 2.3. Mean (\pm SE) biofilm biomass by area (mg dw cm^{-2}) expressed as measurements from samples taken through the pre-exposure and exposure periods (days -7, 0 and 14) for all Se treatments ($0.33 \mu\text{g Se L}^{-1}$ [control], $5 \mu\text{g SeIV/SeVI L}^{-1}$, and $25 \mu\text{g SeIV/SeVI L}^{-1}$), separated by field site (Chris' Pond, Summit Lake, Roadside Lake, and Smeaton Pond). Different letters (a-c) denote statistical significance ($p < 0.05$) relative to other treatment groups.

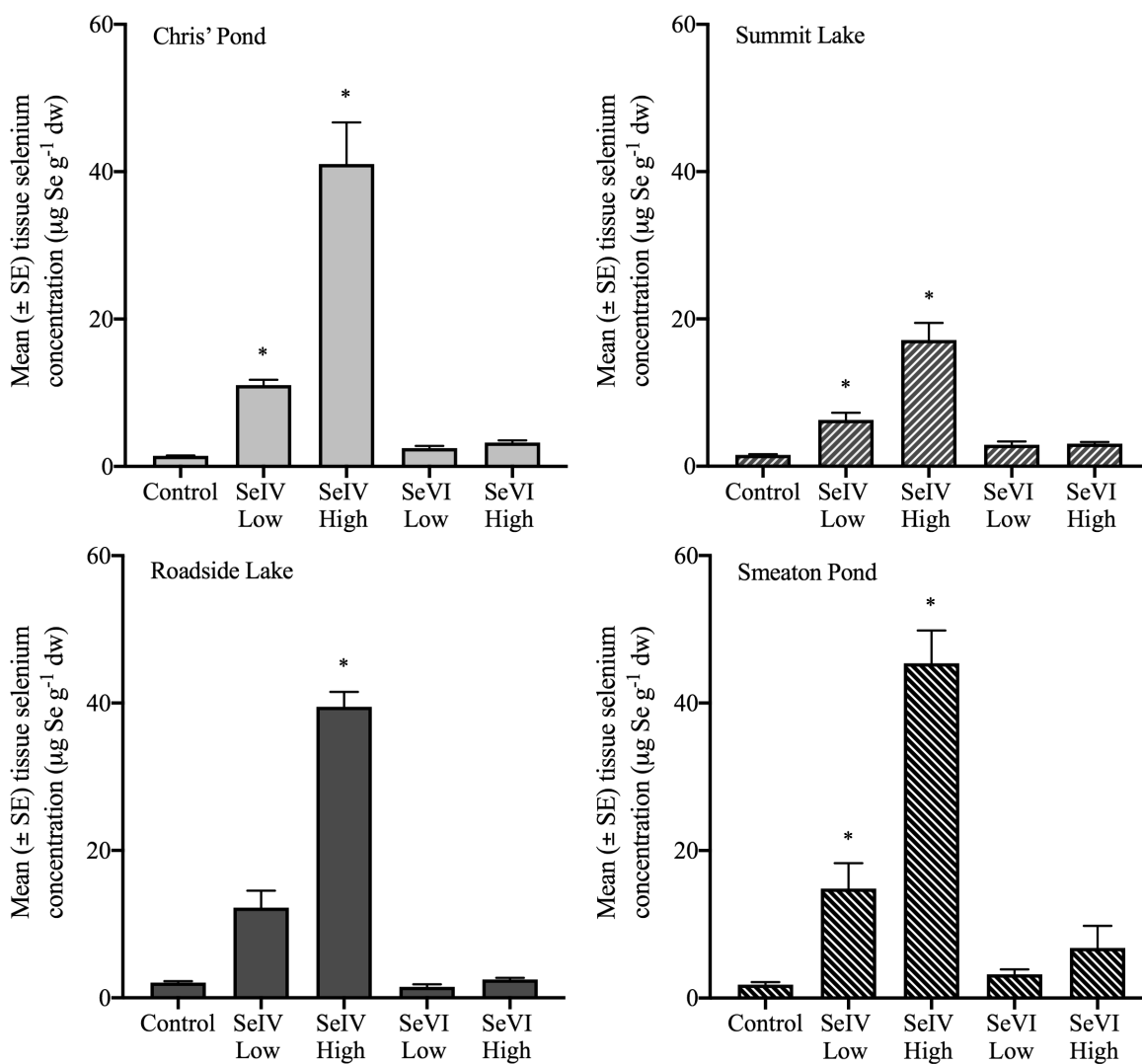


Figure 2.4. Mean (\pm SE) biofilm tissue Se concentrations ($\mu\text{g Se g}^{-1} \text{ dw}$) measured following exposure to respective aqueous Se treatments ($0.33 \mu\text{g Se L}^{-1}$ [control], $5 \mu\text{g SeIV/SeVI L}^{-1}$, and $25 \mu\text{g SeIV/SeVI L}^{-1}$), separated by field site (Chris' Pond, Summit Lake, Roadside Lake, and Smeaton Pond). Values were represented by the mean concentrations measured on sampling days 0 and 14 of exposure. An asterisk (*) denotes statistical significance ($p < 0.05$) relative to respective control treatments.

were 2.4-fold lower than those measured among all other sites of the same treatment. Roadside Lake biofilms only showed a statistically significant increase in the high SeIV treatment relative to the controls ($p < 0.01$), though a similar concentration-dependent trend was present across increasing SeIV concentrations, where biofilm Se residues averaged $12 \pm 2.3 \text{ Se g}^{-1} \text{ dw}$ and 40 ± 2.0 in the low and high SeIV treatments, respectively. As a result, enrichment functions (EF) were similar across all sites, with the exception of Summit Lake, which had the lowest enrichment at $1,477 \pm 224$ and 817 ± 110 in the low and high SeIV treatments, respectively, in comparison to the higher enrichment averaging $2,519 \pm 426$ and $1,742 \pm 169$ in the same respective treatments across the other three collection sites (Table A.2.3).

Relative to Se bioconcentration observed in the SeIV treatments, Se accumulation into biofilms in the SeVI treatments was not significant. Smeaton Pond biofilms in the low SeVI treatment showed the greatest enrichment among field sites, with a mean tissue Se concentration of $3.2 \pm 0.7 \text{ } \mu\text{g Se g}^{-1} \text{ dw}$, translating to a 1.8-fold increase relative to the control. Smeaton Pond biofilms also showed the greatest increase in the high SeVI treatment, with a mean tissue Se concentration of $6.8 \pm 3.0 \text{ } \mu\text{g Se g}^{-1} \text{ dw}$, translating to a 3.8-fold increase relative to the control.

2.4.3 Trophic transfer of selenium from field-grown biofilm to *H. azteca* and amphipod growth

In contrast to the similarities in trends observed in the bioconcentration of Se into the primary producer biofilms, the trophic transfer of Se into the amphipods exposed through diets of the site-specific biofilms followed more variable patterns. Final measured total Se in the bodies of *H. azteca* showed statistically significant differences within treatments from three field sites: Chris' Pond, Summit Lake, and Roadside Lake (K-W: $H=18 - 22$, $p \leq 0.01$) (Figure 2.5). Within the SeIV treatments from these sites, bioaccumulation of Se into amphipods followed a

concentration-dependent relationship, where elevated SeIV was observed in both low and high SeIV treatments relative to respective controls ($p \leq 0.04$). Exposures of *H. azteca* to diets of the high SeIV treatment reached tissue concentrations up to $5.8 \pm 0.8 \mu\text{g Se g}^{-1} \text{ dw}$ in Summit Lake samples, Chris' Pond and Roadside Lake tissues reached 11 ± 0.8 and $18 \pm 3.6 \mu\text{g Se g}^{-1} \text{ dw}$, respectively. Resulting TTFs of Se from the biofilms into amphipods were similar among SeIV treatments for the three aforementioned sites, ranging from 0.46 ± 0.08 to 0.51 ± 0.14 and 0.25 ± 0.03 to 0.50 ± 0.14 in the low and high treatments, respectively, whereas trophic transfer from biofilms collected from Smeaton Pond was comparably lower, with TTFs measured at 0.22 ± 0.05 and 0.15 ± 0.03 for the same respective treatments (Table A.2.4). In comparison, there was no statistically significant difference in Se bioaccumulation into the amphipod tissues of the SeVI treatments for all four sites.

Analysis of the mean growth of *H. azteca* across all treatments through the period of Se exposure resulted in statistical differences when comparing respective site-specific biofilms (K-W: $H=43$, $p < 0.01$) (Fig 2.6). Amphipods exposed to biofilms from Chris' Pond and Roadside Lake saw the greatest growth with mean growth per individual over the 14 days of exposure of $0.20 \pm 0.01 \text{ mg dw}$ and $0.18 \pm 0.01 \text{ mg dw}$ ($p \leq 0.03$), respectively. This growth translated to 2.1- and 2.5-fold increases in mass compared to respective day zero amphipods for Chris' Pond and Roadside Lake. Amphipods exposed to Smeaton Pond biofilms displayed the least growth with a mean of $0.06 \pm 0.01 \text{ mg dw}$, corresponding to the lowest fold change compared to amphipod masses at day zero at 1.6-fold.

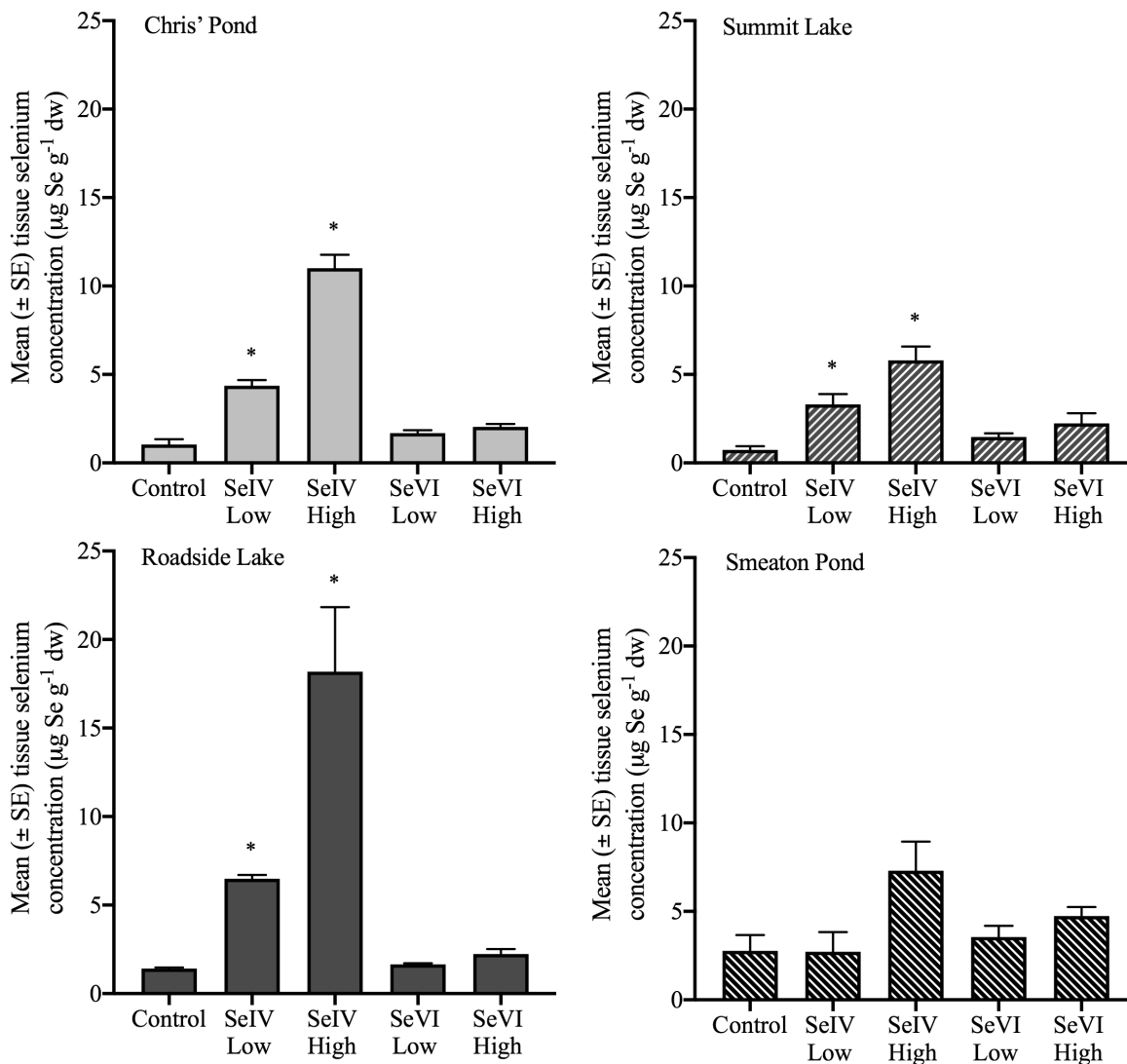


Figure 2.5. Mean (\pm SE) *H. azteca* whole-body Se concentrations ($\mu\text{g Se g}^{-1}$ dw) measured after 14 days of exposure to diets of respective Se-exposed biofilms ($0.33 \mu\text{g Se L}^{-1}$ [control], $5 \mu\text{g SeIV/SeVI L}^{-1}$, and $25 \mu\text{g SeIV/SeVI L}^{-1}$) with concurrent aqueous exposure to corresponding aqueous Se concentrations, separated by field site (Chris' Pond, Summit Lake, Roadside Lake, and Smeaton Pond). An asterisk (*) denotes statistical significance ($p < 0.05$) relative to respective control treatments.

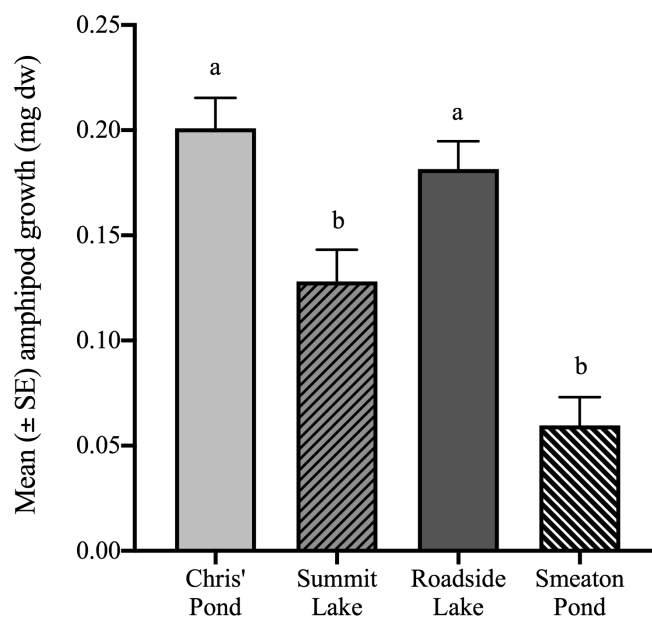


Figure 2.6. Mean (\pm SE) *H. azteca* growth (mg dw) across all treatments measured after 14 days of exposure to diets of site-specific Se-exposed biofilms ($0.33 \mu\text{g Se L}^{-1}$ [control], $5 \mu\text{g SeIV/SeVI L}^{-1}$, and $25 \mu\text{g SeIV/SeVI L}^{-1}$) and concurrent aqueous exposure to corresponding aqueous Se concentrations, separated by field site (Chris' Pond, Summit Lake, Roadside Lake, and Smeaton Pond). Different letters (a-b) denote statistical significance ($p < 0.05$) relative to other treatment groups.

2.5 Discussion

The task of setting Se guidelines protective of aquatic wildlife across the breadth of potential ecological conditions has proven difficult due to idiosyncrasies in the biokinetic characteristics of this element. Despite the knowledge that the greatest bioconcentration step for Se from the aqueous phase occurs at the base of the food web (Stewart et al. 2010), influencing factors on the uptake of Se into this highly impactful lower trophic level in freshwater ecosystems remain poorly studied. Bioaccumulation of Se is expected to vary as a function of species-specific uptake rates, chemical speciation and concentration, and the water chemistry of the surrounding environment (Bowie et al. 1996). The present study found similarities in the bioconcentration of Se among biofilms; however, variability in primary producer biofilm composition and biomass appeared to be influencing factors in trophic transfer of Se into primary consumers. Differences in trophic transfer efficiency to consumers occurred despite similar Se residues measured in the majority of biofilms. The influence of both aqueous and dietary uptake into invertebrate tissues was also consistent with previous publications where uptake from the aqueous phase occurs in addition to dietary accumulation (Besser et al. 1993; Franz et al. 2011), an important finding considering prevailing assumptions of less than 5% of Se accumulation into most invertebrates occurring from the aqueous phase (Presser & Luoma 2010).

2.5.1 Enrichment and trophic transfer of selenium from *S. bacillarus* to *H. azteca*

Amphipod Se residues did not appear to steady state concentrations. This trend was apparent in all elevated SeIV treatments, with the exception of the low concentration without aqueous exposure, which may be indicative of equilibrium within this lowest treatment concentration. Concurrent aqueous Se exposures also appeared to account for greater than 40 % of additional uptake into the primary consumers in comparison to the selenized diet alone.

Continued Se uptake over a multi-week exposure scenario and the contribution of aqueous Se intake in the body burden of an invertebrate are important considerations for modeling Se exposure scenarios with variable exposure duration and inputs. The rate of uptake of dissolved Se has been previously regarded as slow in comparison to uptake from dietary sources and therefore irrelevant to modeling (Presser & Luoma 2010). However, this conclusion is largely based on data collected from marine and estuarine species (Luoma et al. 1992; Schlegel et al. 2002; Wang 2002) or freshwater species that were exposed for time periods not representative of real-world scenarios (Roditi et al. 2000; Lee et al. 2006). Though the initial hours of Se exposure may represent the greatest relative influx of dissolved Se into invertebrates, time to reach steady-state concentrations involves an extended exposure period over several days, varies by chemical species, and represents a significant portion of overall Se accumulation (Besser et al. 1993; Gallego-Gallegos et al. 2013), consistent with the results of the present study. Given the potential for prolonged exposure to aqueous-borne Se species *in situ*, both aqueous and dietary pathways may need to be considered to model Se uptake into primary consumer invertebrates.

2.5.2 Field-grown biofilm algal identification and enrichment of selenium

Field collection of natural biofilms from water bodies with different general water qualities successfully yielded different assemblages of primary producers. Despite composition differences, concentration-dependent trends in the bioconcentration of SeIV were consistent across biofilms. The most productive biofilm by biomass showed reduced overall Se accumulation, resulting in lower dietary concentrations exposed to higher trophic levels. A reduced surface area may be responsible for the difference observed in this biofilm, where increased biofilm thickness may have altered the Se enrichment capacity within the biofilm. In conjunction with known competitive interactions caused by ionic composition (Ogle & Knight 1996; Yu & Wang 2004a, b; Lo et al.

2015), these results would suggest that the biokinetics of Se in aquatic environments could be further predicted based on primary producer productivity and biomass.

At similar exposures of aqueous inorganic Se, SeIV accumulation into primary producers was far greater than that of SeVI. This finding is consistent with previous research outcomes, showing SeIV as the chemical species of inorganic Se most available to algae (Riedel et al. 1991; Hu et al. 1997; Markwart et al. 2019). In contrast, the limited uptake of SeVI into the biofilms is greatly reduced compared to that of Markwart et al. (2019), and believed to be largely attributable to background levels of sulfate (SO_4) in test waters (Saskatoon municipal water: $86 \pm 6.1 \text{ mg SO}_4 \text{ L}^{-1}$). Reduced assimilation of SeVI has been recorded in test systems with elevated ratios of SO_4 :SeVI, a result observed in both primary producers (Williams et al. 1994; Lo et al. 2015) and consumers (Hansen et al. 1993; Ogle and Knight 1996; Brix et al. 2001). Due to the structural similarity between SeVI and SO_4 , inhibition of SeVI uptake is believed to be related to competition for common membrane carrier(s) (Hansen et al. 1993). SO_4 -dependent SeVI screening guidelines have been previously recommended based on a compilation of data from laboratory studies and validation using field data (DeForest et al. 2017). In comparison to the DeForest et al. (2017) screening guideline of $21 \text{ } \mu\text{g SeVI L}^{-1}$ for $100 \text{ mg SO}_4 \text{ L}^{-1}$ (for a lotic system), the present test systems contained higher waterborne concentrations of $25 \text{ } \mu\text{g SeVI L}^{-1}$ and lower background SO_4 . This resulted in Se bioaccumulation into aquatic invertebrate tissues below the British Columbia Ministry of Environment guideline of $4 \text{ } \mu\text{g g}^{-1} \text{ dw}$ (BC MoE 2014), with the exception of one site where reduced relative amphipod growth may have been a factor in elevated Se concentrations.

In comparison to the work done by Markwart et al. (2019), where Se bioconcentration was investigated in biofilms collected from identical field-sites as the present study, total apparent biofilm uptake differed in some instances depending on Se treatment and field site. Uptake

measured in the Markwart et al. (2019) biofilms was higher under most conditions compared to the present study. The greatest difference in uptake of Se between studies occurred in the low SeIV treatments from Smeaton Pond, where biofilm residues measured in the referenced study were 8.0-fold greater than the present study. This dissimilarity also occurred in the high SeIV, showing a 4.9-fold relative increase in the referenced studies. Markwart et al. (2019) postulated that the greatly increased Se measured in Smeaton Pond biofilms of the SeIV treatments were the result of notably high iron at this site, which was predicted to manifest in the form of precipitates associated with the extracellular matrices of biofilms (Letovsky et al. 2012). Iron oxyhydroxides are known to have a high affinity for SeIV (Balistrieri & Chao 1990), adsorbing SeIV to the biofilms, resulting in an apparent elevation in measured Se. This relationship has been reported to be highly pH-dependent, where SeIV adsorption increases with decreasing pH (Balistrieri & Chao 1990). The comparably low Se bioconcentration measured in Smeaton Pond biofilms in the present study may be explained by the difference in test water pH between studies, pH values deviating one pH unit between studies (7.5 – 8.3), a range that has demonstrated pH-dependent adsorption of SeIV (Balistrieri & Chao 1990). Where Markwart et al. (2019) also saw concentration-dependent uptake of SeVI into field-collected biofilms, this finding did not occur in the present study. The reason for this discrepancy may be explained by differences in background ion concentrations in respective test waters. Specifically, dissolved SO_4 was present in comparably low concentrations ($5.7 \text{ mg SO}_4 \text{ L}^{-1}$) in the growth medium used in Markwart et al. (2019) (ECCC 2007).

2.5.3 Trophic transfer of selenium from field-grown biofilm to *H. azteca* and amphipod growth

Beyond the investigated uptake of Se into field-collected primary producer biofilms, trophic transfer of the bioconcentrated Se into a primary consumer representative of Canadian

cold, freshwater ecosystems was examined. Whole-body Se concentrations observed in the *H. azteca* exposures support the concept that Se taken up by primary producers at the base of the food web is transferred to primary consumers primarily via the diet; however, aqueous uptake also contributed more than 40% of Se to the amphipod body burdens after 15 days. Differences in relative uptake into the food chains occurred through exposure to variations in dietary community composition and biomass. Measured residues in amphipods generally followed a concentration-dependent relationship in the SeIV treatments, and negligible Se uptake from exposure to the SeVI treatments was observed, similar to the biofilm trophic step. Under some of the presented experimental scenarios, exposure of amphipods to low or high Se treatments resulted in whole-body Se concentrations that exceeded the BC MoE (2014) dietary aquatic invertebrate tissue guideline of $4 \mu\text{g g}^{-1} \text{ dw}$. This guideline serves primarily as a trigger for further investigation in scenarios of elevated Se and is intended for the protection of higher consumer species. TTFs of Se from the dietary biofilms into the consumer amphipods varied to some extent with differing treatment scenarios, though they were similar to previously reported values for a related marine amphipod (*L. plumulosus*: Assimilation efficiency = 32 – 70 % from 5 phytoplankton species) (Schlekat et al. 2002).

Trophic transfer of Se into the amphipods was comparable when dietary exposures involved similarly structured algal compositions. Biofilms dominated by chlorophytes showed the greatest resulting Se residues in the consumer species. The relative differences in the assimilation of Se from the different biofilms could be caused by a number of factors. Comparatively lower uptake of Se was observed in treatments with a combination of proportional biofilm dominance by diatoms and higher overall biofilm biomass or an increased proportion of cyanophytes in the biofilm's community composition. The increased proportion of cyanophytes in treatments from

Smeaton Pond may have contributed to the observed reduced uptake, as assimilation efficiency of cyanophytes into the diet of *H. azteca* is significantly reduced in comparison to other bacteria, diatoms and green algae species (Hargrave 1970). A high proportion of unsuitable dietary constituents in the Smeaton Pond biofilm may explain the reduced growth of amphipods due to selective grazing on this biofilm compared to the other biofilms (Brett et al. 2006). This might explain the higher levels of Se measured in the Smeaton Pond controls relative to other field sites due to a lack of growth dilution occurring in Smeaton Pond amphipods. A growth dilution effect with increased food rations has been previously reported, with reduced dietary biomass associated with a lower body mass and higher tissue Se concentration in invertebrates (Conley et al. 2011). The combined influence of differential Se bioconcentration by biofilm constituents and selective grazing by consumers on these constituents remain important unresolved factors potentially affecting final Se concentrations among all exposure scenarios.

2.5.4 Conclusion

The objectives of the present research were to evaluate the influence of primary producer community compositions on the enrichment and trophic transfer of dissolved inorganic Se into a primary consumer species. Variability in resulting whole-tissue Se concentrations in both the primary producer and consumer trophic levels was likely influenced by key experimental parameters. Higher biofilm biomass resulted in lower overall Se enrichment. Reduced growth in the primary consumer was hypothesized to have been associated with an increased presence of cyanophytes. Concurrent exposure to dissolved and dietary Se resulted in greater accumulation in the primary consumer than dietary exposure alone. As previously recorded in peer-reviewed literature, the presence of SO_4 in test waters likely resulted in an antagonistic interaction with the SeVI treatments. Such factors may have thereby reduced the concentration of Se reaching the

higher trophic level. Further data associated with Se bioconcentration in primary producers is paramount to a thorough understanding of Se biokinetics, particularly given the limited data related to variability and the knowledge that it represents the greatest contribution to Se accumulation. Together, the influencing characteristics identified by the present research, namely periphytic community composition and biomass, organism autecology, and water quality characteristics, represent important considerations for the management of Se, particularly as potential contributing factors for the creation of models for site-specific Se guidelines.

CHAPTER 3

ENRICHMENT AND TROPHIC TRANSFER OF SELENITE UNDER LABORATORY CONDITIONS: FROM A PRIMARY PRODUCER THROUGH TO A SECONDARY CONSUMER

Preface

The research in this chapter was designed to assess the enrichment of inorganic selenium, as selenite, into a representative primary producer, and its trophic transfer through to a primary consumer *Hyalella azteca* and a secondary consumer *Pimephales promelas*. In conjunction with the research in Chapter 2, this study aimed to extend the simulated aquatic food chain to include an oviparous vertebrate. This chapter will be submitted to the journal Aquatic Toxicology. The anticipated citation is: Raes, K.A., Liber, K., Janz, D., Doig, L.E., Lane, T., Bluhm, K., Green, D., & Hecker, M. (2020). Enrichment and trophic transfer of selenite under laboratory conditions: from a primary producer through to a secondary consumer. *Aquat Toxicol*, (in preparation).

The author contribution to chapter 2 of this thesis were as follows:

Katherine Raes (University of Saskatchewan) designed the study, collected, processed, and analyzed all samples, performed all statistical analyses, and drafted the manuscript.

Karsten Liber (University of Saskatchewan) helped design the study, provided scientific input and guidance, reviewed and revised the manuscript, and provided comments and corrections, and procured and provided funding required to conduct the research.

David Janz (University of Saskatchewan) helped design the study, provided scientific input and guidance, reviewed and revised the manuscript, and provided comments and corrections, and procured and provided funding required to conduct the research.

Lorne Doig (University of Saskatchewan) helped design the study, provided scientific input and guidance, reviewed and revised the manuscript, providing comments and corrections.

Taylor Lane (University of Saskatchewan) helped design the study and provided scientific input.

Kerstin Bluhm (University of Saskatchewan) helped design the study and provided scientific input.

Derek Green (University of Saskatchewan) helped design the study and provided scientific input.

Markus Hecker (University of Saskatchewan) helped design the study, provided scientific input and guidance, reviewed and revised the manuscript, providing comments and corrections, and procured and provided funding required to conduct the research.

3.1 Abstract

The influence of biogeochemistry on the uptake and transformation of Se at the base of the aquatic food chain, and its subsequent influence on trophic transfer to higher level consumers such as fish, remains unsettled. Therefore, the purpose of this study was to determine the efficiency of selenite (SeIV) bioconcentration and trophic transfer from a primary producer to a primary consumer, and finally to a representative secondary consumer species under controlled laboratory conditions, simulating a simple freshwater food chain. Green algae, *Stichococcus bacillarus*, were exposed to multiple aqueous SeIV concentrations and subsequently fed to the primary consumer *Hyaella azteca*. Uptake was quantified and three resulting *H. azteca* dietary treatments (Control [$1.6 \mu\text{g Se g}^{-1} \text{ dw}$], low [$6.9 \mu\text{g Se g}^{-1} \text{ dw}$], and high [$19 \mu\text{g Se g}^{-1} \text{ dw}$]) were fed to the fish, *Pimephales promelas*, in a partial lifecycle reproductive assay. Compartmental distributions of Se into adult fish tissues, reproductive output, and maternal transfer into the offspring were measured. All dissolved SeIV treatments were above the Canadian water quality guideline (MMER: $1 \mu\text{g Se L}^{-1}$), and yielded Se concentrations in amphipods above a Canadian dietary invertebrate tissue guideline ($4 \mu\text{g Se g}^{-1} \text{ dw}$) (BC MoE 2014). Adult fish and embryos remained below respective tissue Se concentration guidelines (BC MoE Guidelines: ovaries/eggs - $11 \mu\text{g Se g}^{-1} \text{ dw}$; muscle - $4 \mu\text{g Se g}^{-1} \text{ dw}$) in fish fed the low SeIV diet and exceeded these guidelines when fed the high SeIV diet. Fish embryo Se concentrations reached up to $17 \mu\text{g g}^{-1} \text{ dw}$ in the high SeIV treatment, and final mean adult female tissues measured 4.5 ± 1.1 , 16 ± 1.8 and $17 \pm 1.9 \mu\text{g Se g}^{-1} \text{ dw}$ in muscle, gonads and livers, respectively. Reproductive success showed a negative trend in the elevated Se treatments, and resulting incidence of fish fry deformities in reared F1 offspring was greatest in the low SeIV treatment. This study will serve to reduce uncertainty in Se trophic transfer

as it relates to dosing methodology, critical for informing regulatory practices in the management of elevated Se in the environment.

3.2 Introduction

Selenium (Se) has become a contaminant of particular concern in North America because it can cause population-level declines in sensitive oviparous vertebrates at elevated aqueous concentrations. Elevated levels of Se contamination occur primarily as a result of anthropogenic activities, often involving the mobilization of subterranean resources, particularly those associated with geologic formations of organic-rich marine basins (Presser et al. 2004a). These include mining activities, fossil fuel combustion, oil refining, fertilizer production, and agricultural irrigation (Cumbie and Van Horn 1978; Cutter 1991; Muscatello & Janz 2009). Multiple scenarios have become classic examples of ecosystem disruption involving elevated Se exposure. These include mortality and reproductive failure in fishes resident to Belews Lake (Cumbie & Van Horn 1978) and Hyco Lake (Crutchfield 2000) in North Carolina, USA in the 1970s, and embryo mortalities in aquatic birds (Ohlendorf et al. 1986) and local extirpation of multiple fish species (Saiki & Lowe 1987) inhabiting the seleniferous agricultural drainwater of the Kesterson Reservoir in California, USA in the 1980s. The propensity for inorganic Se species in the aquatic environment to be efficiently bioconcentrated by primary producers and transferred to higher trophic levels via dietary pathways has regulatory agencies searching for appropriate risk characterization and monitoring strategies (Lemly & Skorupa 2007; Presser & Luoma 2010; DeForest et al. 2017).

Dissolved Se is effectively bioaccumulated and biotransformed by aquatic microorganisms into organo-Se compounds, which are readily transferred to higher trophic levels primarily through dietary pathways (Stewart et al. 2004; Janz et al. 2014). In fact, the diet represents the principal

pathway for Se exposure, transfer, and toxicity in aquatic ecosystems to higher, sensitive organisms of primary concern, chiefly oviparous vertebrates such as fish and birds (Stewart et al. 2010). Uptake of Se at the base of the aquatic food web by primary producers (e.g., algae and macrophytes) and microorganisms (e.g., bacteria and fungi) comprises the largest contribution to its bioconcentration (Luoma & Presser 2009). It also represents a great source of uncertainty regarding the magnitude of trophic transfer to higher organisms. Significant knowledge gaps exist regarding kinetic processes involved in the enrichment of Se and the examination of taxon-specific differences in uptake by a diversity of primary producers (Stewart et al. 2010). Bioaccumulation of Se in an aquatic ecosystem varies as a function of species-specific uptake rates, Se speciation, concentration of the element, and water chemistry of the receiving environment; however, the relative influence of these characteristics remains uncertain (Bowie et al. 1996). As a result of this diet-driven bioaccumulation and uncertainties associated with enrichment at the base of the food web, elevated levels of Se in aquatic environments have become intrinsically associated with ecological risk and necessary management.

Inorganic Se is released by anthropogenic activities predominantly in the form of selenite (+4 oxidation state; SeIV) or selenate (+6 oxidation state; SeVI) oxyanions, depending on the source and processing of the material (Maher et al. 2010). In general, our understanding of the uptake and biotransformation of these Se oxyanions is limited, particularly in regard to aquatic primary producers, which are expected to drive Se biogeochemical cycling in aquatic environments (Cutter & Bruland 1984). Though inorganic Se species are dominant in the aqueous environment, the biotransformation and trophic transfer of organic species through the food web has been hypothesized to be largely responsible for governing the element's toxicity to higher animals (Presser & Luoma 2009). However, due to the influence of multiple abiotic and biotic

factors, the total concentration of Se in any environmental compartment has a limited capacity to predict toxicity. The fate and bioavailability of Se are governed by many factors, including Se speciation, water chemistry, and the capacity for biotransformation in the receiving environment (Stewart et al. 2010).

Reported adverse effects caused by Se exposure have involved numerous systemic processes in oviparous vertebrates, particularly in fish species (Hilton et al. 1980; Hunn et al. 1987; Ogle & Knight 1989). This is likely due in part to differences in species, life stage, exposure route, and other factors that differed among studies. Teratogenicity in oviparous animals has been a consistent bioindicator of Se exposure, occurring at lower exposure concentrations than acute mortality in adults (Ohlendorf et al. 1986; Presser 1994). A significant body of evidence confirms the existence of Se residues in the eggs and embryos of reproductively-impacted oviparous vertebrate populations (Cumbie and Van Horn 1978; Ohlendorf 1989; Stewart et al. 2004; Muscatello et al. 2006). The primary mechanism by which Se causes teratogenicity has been debated, though there is increasing evidence supporting the role of oxidative damage as the initiating event in embryo mortality and teratogenicity (Hoffman 2002; Holm 2002).

Numerous laboratory toxicity tests have been conducted with the purpose of quantifying toxic concentration thresholds in particularly sensitive taxa (Gillespie & Baumann 1986; Skorupa & Ohlendorf 1991; Hermanutz et al. 1992; Masse et al. 2015). Dietary Se toxicity to freshwater fishes are most commonly evaluated in the laboratory with selenomethionine-spiked (SeMet) feed (Hamilton et al. 1990; Hardy et al. 2010; Thomas & Janz 2011). Quantification of Se speciation has considerably improved in recent years, with organoselenides being confirmed as the dominant class present in biota exposed to elevated concentrations of Se, and SeMet being regarded as the major chemical species (Gallego-Gallegos et al. 2013; Conley et al. 2013; Schmidt et al. 2013).

The assumption of SeMet being the dominant Se species driving Se toxicity has been the basis for the majority of studies conducted to assess Se toxicity. However, this experimental assumption has been contested due to conclusions being drawn from exposures not being representative of naturally occurring Se mixtures, as well as differences that might result from exposure to free SeMet in comparison to what would be protein-bound in nature (Rigby et al. 2014).

The present research contributes to the understanding of Se tissue compartmentalization and reproductive success resulting from dietary Se exposures to fish, and identifies potential differences occurring between artificially-dosed and naturally-enriched Se diets. Enrichment of dissolved inorganic SeIV into primary producers, and trophic transfer to primary and secondary consumers were measured. Resulting reproductive output, Se maternal transfer and deformities in the secondary consumer offspring were also evaluated. Objectives of the present study were to characterize enrichment and multi-level trophic transfer of inorganic Se from the aqueous phase into a simple, environmentally relevant, freshwater food chain.

3.3 Materials and methods

3.3.1 Diet collection

Diets were generated through multiple stages of trophic exposure to mimic the ecological occurrence of Se within a food web, where primary producers were exposed to aqueous inorganic SeIV, and were then fed to a primary consumer. The primary producer, *Stichococcus bacillarus*, was sourced from an in-house laboratory monoculture originally acquired from the Canadian Phycological Culture Centre (CPCC) at the University of Waterloo, Waterloo, ON, Canada. The primary consumer, the amphipod species *Hyaella azteca*, was also acquired from an in-house culture in the Toxicology Centre at the University of Saskatchewan, SK, Canada.

The algae were maintained following guidance from the Environment Canada Biological

Test Method for Growth Inhibition in Freshwater Alga (ECCC 2007). Specifically, the *S. bacillarus* test culture was maintained in Bold's Basal Medium for green algae (Stein et al. 1973) at an ambient temperature of $24 \pm 1^\circ\text{C}$ under a 16:8 hour light:dark photoperiod in an incubator. Subsamples of *S. bacillarus* were exposed to aqueous NaSeO_3 for eight days at nominal aqueous Se concentrations of 0 (control), 9, 27 and $54 \mu\text{g Se L}^{-1}$. Aqueous exposures were refreshed every two days, with a third replacement of the dosed growth medium. On the eighth day of exposure, algae were separated from the aqueous exposure through centrifugation at 1800 g for 10 min, with a mean (\pm SE) mass of 0.7 ± 0.02 g ww per 50-mL vessel, and stored in a refrigerator at 4°C . *Hyaella azteca* were maintained following guidance from the Environment Canada *Hyaella azteca* Biological Test Method for Survival and Growth in Sediment and Water (ECCC 2013) in dechlorinated Saskatoon municipal water with bromine supplementation at an ambient temperature of $24 \pm 1^\circ\text{C}$ under a 16:8 hours light:dark cycle in a controlled environmental chamber. Amphipods were reared in 19.8-L HDPE containers, containing 16 L of aerated water, 1 kg of silica sand, and 400 cm^2 rinsed cheesecloth substrate. To mimic a more probable ecological contamination event, aqueous SeIV exposure concentrations equivalent to the respective *S. bacillarus* exposures were maintained during rearing of the selenized amphipods (0.3 [control], 9, 27 and $54 \mu\text{g Se L}^{-1}$). Selenized algae were resuspended in *H. azteca* exposure water at the respective SeIV concentrations for feeding to the amphipod, with daily additions of 1 mL algae suspensions. Amphipod subsamples were collected weekly, following an initial four weeks of exposure, to allow time to reach steady state. Amphipods were not gut purged, to represent a diet that would be encountered in the environment. Samples with a mean (\pm SE) mass of 0.5 ± 0.03 g ww were collected in 2-mL microcentrifuge tubes, flash frozen in liquid nitrogen, and stored in a freezer at -20°C until a sufficient mass was acquired for the fish feeding experiment.

3.3.2 Fish exposure and reproduction study experimental setup

The adult fish reproductive assay followed a modified version of methods established by a previous dietary Se fathead minnow reproductive assay (Ogle & Knight 1989) and generalized short-term (21 days) fathead minnow reproduction protocol (Ankley et al. 2001). Minnows were sourced from an in-house culture maintained in the Aquatic Toxicology Research Facility, University of Saskatchewan, SK, Canada. Exposures were conducted in twelve 20-L borosilicate glass aquaria, containing 18 L of aerated dechlorinated municipal water and two 4-inch PVC breeding tiles in a controlled environmental chamber, with an ambient temperature of $25 \pm 1^\circ\text{C}$ under a 16:8 hour light:dark cycle. Two thirds of the water were refreshed daily. Sexually mature fathead minnow adults (6-12 months old), two females and one male, were added to each aquarium. Fish were fed twice daily at 5% body weight per day, transitioning from their usual culture diet of thawed chironomids to a diet containing the control *H. azteca* diet over the second week of acclimation. A week-long baseline period, where all fish were fed the control diet, followed the acclimation period when successful breeding was observed in all twelve aquaria. Embryos were counted daily for the remaining extent of experimentation to assess fecundity. On day zero of Se exposure, aquaria were ranked based on their relative embryo production with the intention of equalizing baseline reproductive output among treatments, and sorted into three treatment groups (control, low SeIV and high SeIV) for a total of four replicates per treatment. For the subsequent 21 days, fish were fed 0.1 ± 0.01 g dw (0.5 ± 0.03 g ww) of their respective treatment amphipod diet, supplemented by 0.06 ± 0.01 g dw (0.9 ± 0.09 g ww) of undosed chironomids to maintain adequate feeding to ensure breeding conditions. Total dietary exposure concentrations fed to adult fish were calculated based on the relative dry weight contribution of dosed amphipods and undosed chironomids for each treatment (Eq. 3.1):

$$\begin{aligned} \text{Total dietary Se concentration} = & | (0.65) \times (\text{amphipod Se concentration } [\mu\text{g Se g}^{-1} \text{dw}]) | + \\ & | (0.35) \times (\text{chironomid Se concentration } [\mu\text{g Se g}^{-1} \text{dw}]) | \end{aligned} \quad (3.1)$$

At the termination of the exposure, adult fathead minnows were humanely sacrificed using percussive stunning followed by spinal dislocation. Morphometric measurements were taken and the fish dissected for analysis. Individual total body mass (g) and standard length (cm) were measured, and the gonad, liver, and muscle were removed and weighed, flash frozen in liquid nitrogen, and stored in a freezer at -20°C for Se analysis. Condition factor (K), liversomatic indices (LSI), and gonadosomatic indices (GSI) were calculated (Eqs. 3.2-3.4):

$$K = 100 \times [\text{total body mass (g)} / \text{standard length (cm)}^3] \quad (3.2)$$

$$LSI = 100 \times [\text{liver mass (g)} / \text{total body mass (g)}] \quad (3.3)$$

$$GSI = 100 \times [\text{gonad mass (g)} / \text{total body mass (g)}] \quad (3.4)$$

3.3.3 Offspring rearing

Throughout the baseline and experimental periods, fish embryos were counted and collected daily. Subsamples were flash frozen in liquid nitrogen, and stored in a freezer at -20°C for later Se analysis. A subsample of 20 embryos from three replicates per treatment were collected after 7, 14 and 21 days of exposure when possible. Embryos were reared in 60 mL dechlorinated water in borosilicate glass petri dishes, with daily half volume water renewals, in an environmental chamber with an ambient temperature of $25 \pm 1^\circ\text{C}$ under a 16:8 hours light:dark cycle. Survival, eye up, and hatching success were recorded daily. After seven days, surviving fry were preserved in 10% buffered formalin for 24 hours, and then transferred to 70% ethanol for later deformities analysis.

3.3.4 Analyses

Subsamples of dosed *S. bacillarus* were collected following the aforementioned isolation methods on day 8 of each algae exposure, and *H. azteca* were subsampled at 5-7, 10, 12, and 16 weeks of the diet collection. *Pimephales promelas* embryos were collected throughout the baseline period and reproductive assay, and dissected adult fathead minnow liver, gonad, and muscle tissues were subsampled at the termination of the reproductive assay. All tissue samples were flash frozen with liquid nitrogen and freeze dried for dry weight total Se analysis. Samples for dissolved Se analysis were collected from a subsample of fish aquaria (n=3) on days 0 and 21, as well as before and after water changes on day 14 in 8-mL HDPE sample bottles using syringe filters (0.45 µm pore size, polyethersulfone membrane, VWR International, Radnor, PA, USA) and acidified with high-purity nitric acid (Optima Grade Nitric Acid, Fisher Scientific, Hampton, NH, USA). Sampling before and after water renewals had the purpose of capturing any variability in dissolved Se, which was found to be negligible. Mean (\pm SE) dissolved total Se concentrations measured in exposure waters are summarized in the appendix (Table A.3.1).

Samples were taken for water quality analysis (Temperature, dissolved oxygen (DO), conductivity, pH, hardness, alkalinity, and ammonia) from a subsample of adult fish test aquaria on days -7, 0, 7, 14, and 21 to ensure consistency throughout experimentation. Temperature, DO, pH, and conductivity were measured with a portable meter (YSI Professional Plus Quatro, Xylem, Yellow Springs, OH, USA), total ammonia was measured using a salicylate-based test kit (API Aquarium Pharmaceuticals, Chalfont, PA, USA), and hardness and alkalinity were measured by titration (Total Hardness Test Kit & Alkalinity Test Kit, LaMotte, Chestertown, MD, USA). Mean (\pm SE) water quality parameters throughout the Se dietary exposure period are summarized in the appendix (Table A.3.1).

All Se analyses were performed at the Toxicology Centre, University of Saskatchewan, SK, Canada using inductively coupled plasma mass spectrometry (8800 ICP-MS Triple Quad, Agilent Technologies, Santa Clara, CA, USA) with a minimum detection limit of $0.06 \pm 0.03 \mu\text{g Se L}^{-1}$. Selenium concentrations in algae, amphipods, and fish were measured following digestions. High purity, 69% nitric acid (HNO_3) (1 mL) and high purity, 30% hydrogen peroxide (0.67 mL) (Sigma Aldrich, St. Louis, MO, USA) were added to teflon digestion vessels with freeze-dried, pre-weighed sample materials. Capped vessels were placed in a MARS-5 microwave digestion system (EM Corporation, Matthews, NC, USA) and held at 160°C for 20 min. Digests were filtered, diluted to 2% HNO_3 , and stored in 8-mL HDPE sample bottles for later Se analysis. Certified reference material (TORT-3 lobster hepatopancreas reference material for trace metals, National Research Council Canada, Ottawa, ON, CA; 1640a trace elements in natural water, National Institute of Standards and Technology, Gaithersburg, MA, USA), duplicates, and method blanks were used to ensure analytical accuracy and validity.

Fish fry were blindly and randomly inspected for deformities under a dissecting microscope (Zeiss Stemi 508, Carl Zeiss Canada, Toronto, ON, Canada). Deformities were recorded as present or absent for each larva. Graduated severity index ratings (scored as normal [0], mild [1], moderate [2], or severe [3]) were used for identified deformities, assessments including skeletal deformities (kyphosis, lordosis and scoliosis), edema, craniofacial and finfold malformations (Lemly 1997; Rudolph 2008; Rickwood et al. 2008; McDonald & Chapman 2009).

3.3.5 Statistical analysis

A one-way ANOVA was used to analyze total larval deformities in the F1 fathead minnow offspring and embryo Se concentrations between days 13-21 ($\alpha=0.05$). Embryo Se concentrations

throughout experimentation and fry deformity incidence relative to measured embryo Se concentrations were analyzed using a linear regression approach ($\alpha=0.05$). Total embryo production during the parental dietary exposure period was analyzed using a linear mixed model. Two-way ANOVAs were employed when analyzing final adult tissue Se concentrations and morphometric parameters ($\alpha=0.05$). Significant differences were further analyzed using Tukey post-hoc tests, and where data showed unequal variance, the Games-Howell post-hoc test was utilized.

3.4 Results

3.4.1 Diet collection

Selenium concentrations in the dietary algae measured 0.1 ± 0.0 , 1.3 ± 0.3 , 2.6 ± 0.4 , and $5.8 \pm 0.6 \mu\text{g Se g}^{-1}$ in the control, low, medium, and high SeIV exposure groups, respectively. Due to periphytic build up inside diet collection exposure containers, Se-spiked green algae diets were not fully representative of the dietary Se exposure to the amphipods. Unknown primary producer species diversity present within the periphytic build up and differences in species-specific Se uptake rates could have modified the Se concentrations received by the amphipods. Primary producer diversity was not analyzed, therefore the potential impact of this uncertainty could not be determined. Maintained aqueous SeIV within the diet collection exposure containers may have also contributed to the whole-body Se of the amphipods, a factor observed in the second chapter of this thesis and reported in other studies (Besser et al. 1993; Gallego-Gallegos et al. 2013).

The low SeIV amphipod treatment showed the most consistent tissue Se concentrations over time, with mean whole-body Se residues measuring $10 \pm 1.3 \mu\text{g Se g}^{-1} \text{ dw}$ across sampling points (Fig A.3.1), 7.7-fold greater than the added dietary algae, and a bioaccumulation factor (BAF = amphipod concentration / aqueous concentration) of 1111 ± 144 compared to the aqueous

concentration they and the dietary green algae were exposed to (nominal: 9 $\mu\text{g Se L}^{-1}$). The moderate SeIV and high SeIV dietary exposures reached mean *H. azteca* Se concentrations of 19 ± 3.2 and $31 \pm 3.1 \mu\text{g Se g}^{-1}$, respectively. Reduced survival was observed in the highest amphipod Se concentration (mean \pm SE amphipod mass collected per week per treatment: control – 5.8 ± 0.1 g; low – 4.5 ± 0.1 g; medium – 3.8 ± 0.1 g; high – 2.2 ± 0.1 g). Overlap with measured whole-body Se was also measured in the medium Se exposed amphipods after 10 weeks ($26 \pm 2.7 \mu\text{g Se g}^{-1}$) and the high SeIV exposure after 4 weeks of diet collection ($31 \pm 3.1 \mu\text{g Se g}^{-1}$). Due to a combination of reduced survival and similarity in whole-body Se, the high SeIV treatment used as the *P. promelas* diet was comprised of *H. azteca* collected from the high amphipod exposure and those collected after the ninth week of exposure from the moderate concentration, for a collective amphipod treatment measuring $29 \pm 2.3 \mu\text{g Se g}^{-1}$ dw. Accounting for the relative contributions of both dietary components (dosed amphipods and undosed chironomids supplementation), mean (\pm SE) total dietary Se treatments were calculated as the following: Control – $1.6 \pm 0.3 \mu\text{g Se g}^{-1}$ dw, Low SeIV – $6.9 \pm 0.8 \mu\text{g Se g}^{-1}$ dw, and High SeIV – $19 \pm 1.5 \mu\text{g Se g}^{-1}$ dw.

3.4.2 Fish tissue selenium residues

Total Se measured in adult *P. promelas* showed a concentration-dependent increase regardless of sex or tissue analyzed (Figure 3.1). Mean liver Se residues reached the highest concentration among the measured tissues, with the greatest uptake observed in female livers, reaching $17 \pm 1.9 \mu\text{g Se g}^{-1}$ dw in the high SeIV treatment (2-way ANOVA: Treatment – $F_{2,18}=86$, $p<0.01$). Liver Se concentrations significantly increased up to 4.3- and 3.7-fold in males and females, respectively, relative to respective controls ($p<0.01$). Comparably, mean gonad Se concentrations showed similar trends across treatment groups and sex to those measured in the

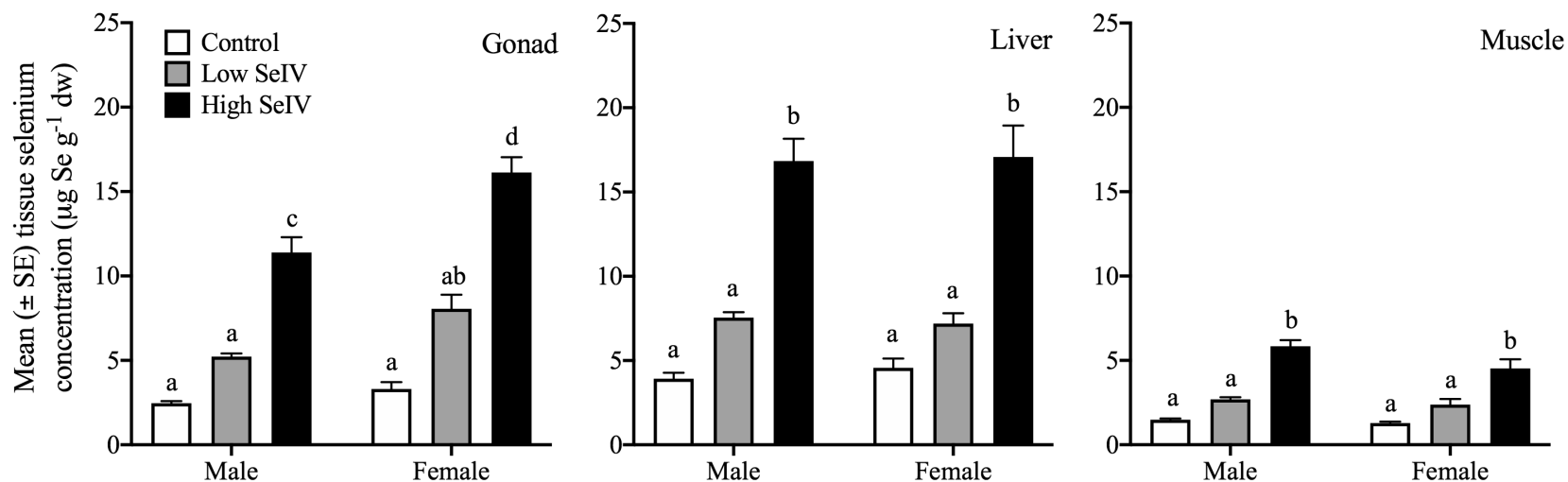


Figure 3.1. Mean (\pm SE) total Se concentrations ($\mu\text{g Se g}^{-1} \text{ dw}$) in three adult *P. promelas* tissues (gonads, liver, and muscle) measured at the termination of exposure to three respective dietary Se treatments. The three dietary treatment groups were Control ($1.6 \mu\text{g Se g}^{-1} \text{ dw}$; white), Low SeIV ($6.9 \mu\text{g Se g}^{-1} \text{ dw}$; grey), and High SeIV ($19 \mu\text{g Se g}^{-1} \text{ dw}$; black). Different letters (a-c) represent a significant difference ($p < 0.05$) in tissue Se concentrations among treatment groups and sexes for each respective tissue.

livers (2-way ANOVA: Treatment*Sex – $F_{2,18}=4.4$, $p=0.03$), though total gonad Se differed between sexes unlike those measured in the livers. In the dosed groups, trophic transfer factors (TTF) were greatest in female gonads of the low SeIV group, reaching 1.2 ± 0.12 in comparison to a transfer efficiency of 0.76 ± 0.03 in the male gonads, though control female livers showed the highest TTFs of all treatments and tissues at 2.8 ± 0.33 (Table 3.1). Final muscle Se concentrations were the lowest of the tissues, somewhat greater levels consistently measured in males relative to females, measuring 5.8 ± 0.7 and $4.5 \pm 1.1 \mu\text{g Se g}^{-1} \text{ dw}$ in the high SeIV treatment, respectively (2-way ANOVA: Treatment – $F_{2,18}=80$, $p<0.0001$; Sex – $F_{1,18}=5.8$, $p=0.03$), corresponding to TTFs of 0.30 ± 0.02 and 0.24 ± 0.03 .

A statistically significant difference was observed between the slopes of measured *P. promelas* embryo Se concentrations relative to time among treatment groups (Linear regression: $F_{2,33}=12$, $p<0.01$). Measured total Se in both the low SeIV and high SeIV treatment embryos increased as time elapsed (Figure 3.2). Between days 0-12 of dietary Se exposure, significant differences in slopes were observed in the low SeIV (slope=0.32, y-intercept=1.84, $p<0.01$) and high SeIV (slope=0.83, y-intercept=5.58, $p=0.03$) treatments relative to the control treatment. For the remainder of the exposure period (days 13-21), the slopes of embryo Se concentrations over time in both the low SeIV and high SeIV treatments did not display significantly different slopes relative to the control (slope=0.00, y-intercept=1.45), suggesting steady state was reached. Furthermore, mean embryo Se concentrations measured between days 13-21 showed a significant difference among treatment groups (ANOVA: $F_{2,12}=332$, $p<0.01$) (Figure 3.3), the low SeIV treatment showing significantly higher Se than the controls ($p<0.01$), and the high SeIV treatment showing greater Se concentrations than both lower Se treatment levels respectively ($p<0.01$).

Table 3.1. Mean (\pm SE) Se trophic transfer factors into three adult *P. promelas* tissues (gonads, liver, and muscle) measured at the termination of exposure to three dietary Se treatments (Control [1.6 $\mu\text{g Se g}^{-1}$ dw]), Low SeIV [6.9 $\mu\text{g Se g}^{-1}$ dw], and High SeIV [19 $\mu\text{g Se g}^{-1}$ dw]).

Dietary Treatment	Sex	Trophic Transfer Factors (Mean \pm SE)		
		Muscle	Gonad	Liver
Control (1.6 $\mu\text{g Se g}^{-1}$ dw)	Male	0.90 \pm 0.05	1.5 \pm 0.08	2.4 \pm 0.22
	Female	0.78 \pm 0.05	2.0 \pm 0.24	2.8 \pm 0.33
Low SeIV (6.9 $\mu\text{g Se g}^{-1}$ dw)	Male	0.39 \pm 0.02	0.76 \pm 0.03	1.1 \pm 0.05
	Female	0.35 \pm 0.05	1.2 \pm 0.12	1.1 \pm 0.09
High SeIV (19 $\mu\text{g Se g}^{-1}$ dw)	Male	0.30 \pm 0.02	0.59 \pm 0.05	0.88 \pm 0.07
	Female	0.24 \pm 0.03	0.84 \pm 0.05	0.89 \pm 0.10

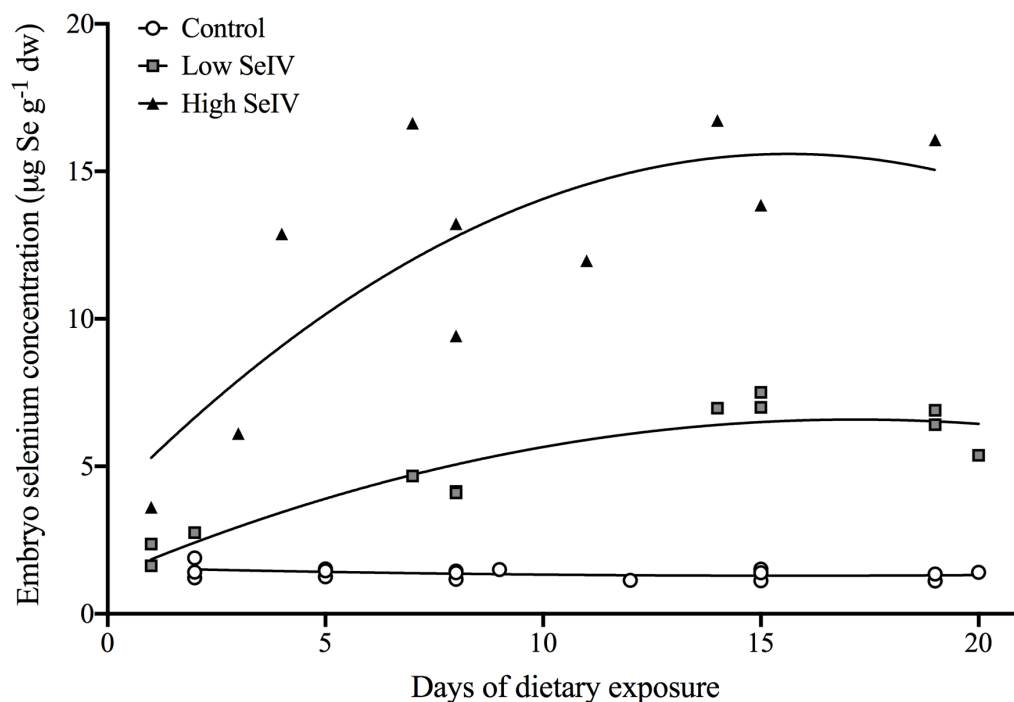


Figure 3.2. Concentration of Se in *P. promelas* embryos ($\mu\text{g Se g}^{-1} \text{ dw}$) during parental exposure to dietary Se. The three treatment groups were Control (white circle) [$1.6 \mu\text{g Se g}^{-1} \text{ dw}$], Low SeIV (grey square) [$6.9 \mu\text{g Se g}^{-1} \text{ dw}$], and High SeIV (black triangle) [$19 \mu\text{g Se g}^{-1} \text{ dw}$]. Each point represents the mean Se concentration measured in a clutch of embryos from an adult breeding group within a treatment group. A line was fitted for each respective treatment group using a second order polynomial ($Y = B_0 + B_1 \cdot X + B_2 \cdot X^2$).

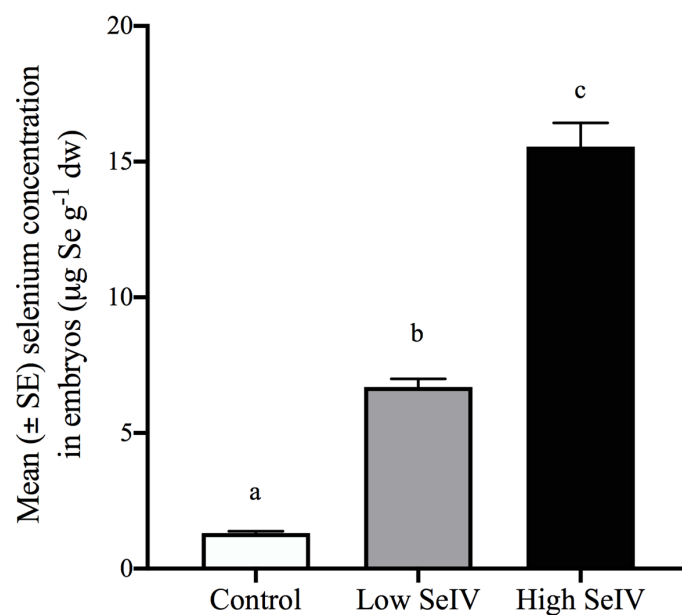


Figure 3.3. Mean (\pm SE) Se concentration of *P. promelas* embryos ($\mu\text{g Se g}^{-1} \text{ dw}$) between days 13-21 of parental exposure to dietary Se. The three treatment groups were Control (white), Low SeIV (grey), and High SeIV (black). Different letters (a-c) represent a significant difference ($p < 0.05$) in Se concentrations among treatment groups.

3.4.3 Biological effects in fish

Measured final adult fathead minnow mass and condition factor both showed similar trends across treatment levels (Table 3.2). Statistical significance was present in both treatment and sex of calculated condition factors of adult fish (2-way ANOVA: Treatment – $F_{2,30}=4.8$, $p=0.015$; Sex – $F_{1,30}=55$, $p<0.0001$). Sex differences were present in total body mass (2-way ANOVA: $F_{1,30}=161$, $p<0.0001$), standard length (2-way ANOVA: $F_{1,30}=75$, $p<0.0001$), LSI (2-way ANOVA: $F_{1,30}=4.6$, $p=0.04$), and GSI (2-way ANOVA: $F_{1,30}=49$, $p<0.0001$). A trend towards an increase in condition and mass occurred in the low SeIV treatment relative to controls, with statistical significance only measured for the condition factors of the females ($p=0.02$).

The total number of embryos produced through the parental dietary exposure period was not significantly different among treatment groups; however, a trend emerged where embryo production decreased with increased dietary Se (Figure 3.4). A statistically significant effect of SeIV treatment was measured in total *P. promelas* fry deformities (ANOVA: $F_{2,21}=7.0$, $p<0.01$) (Figure 3.5). Mean (\pm SE) percent total fry deformities observed in the controls was 23 ± 2.0 %. The low SeIV treatment percent fry deformities significantly differed from control at 37 ± 3.0 % ($p<0.01$). The high SeIV treatment showed no statistically significant difference in fry deformities when compared to the control, with mean (\pm SE) total percent fry deformities of 29 ± 4.0 %. No significant correlation was measured in total deformities of *P. promelas* fry relative to measured embryo tissue Se concentrations (Figure 3.6).

Table 3.2. Mean (\pm SE) morphometric parameters of adult *P. promelas* measured at the termination of exposure to three respective dietary Se treatments (Control, Low SeIV and High SeIV). Different letters (a-b) represent a significant difference ($p < 0.05$) in morphometric measurements among treatment groups for each respective sex.

Dietary Treatment	Sex	Standard Length (cm)	Mass (g)	Condition Factor (K)	LSI	GSI
Control (1.6 $\mu\text{g Se g}^{-1}$ dw)	Male	5.4 \pm 0.3 ^a	3.6 \pm 0.5 ^a	2.3 \pm 0.0 ^a	2.0 \pm 0.5 ^a	0.9 \pm 0.3 ^a
	Female	4.7 \pm 0.1 ^a	1.7 \pm 0.1 ^a	1.7 \pm 0.1 ^a	2.5 \pm 0.3 ^a	8.4 \pm 1.5 ^a
Low SeIV (6.9 $\mu\text{g Se g}^{-1}$ dw)	Male	5.4 \pm 0.1 ^a	3.8 \pm 0.2 ^a	2.4 \pm 0.1 ^a	2.0 \pm 0.2 ^a	0.7 \pm 0.1 ^a
	Female	4.6 \pm 0.1 ^a	1.9 \pm 0.0 ^a	2.0 \pm 0.1 ^b	2.8 \pm 0.2 ^a	9.6 \pm 1.1 ^a
High SeIV (19 $\mu\text{g Se g}^{-1}$ dw)	Male	5.3 \pm 0.1 ^a	3.3 \pm 0.2 ^a	2.2 \pm 0.1 ^a	1.8 \pm 0.2 ^a	0.9 \pm 0.1 ^a
	Female	4.5 \pm 0.1 ^a	1.6 \pm 0.1 ^a	1.7 \pm 0.1 ^{ab}	2.2 \pm 0.4 ^a	9.1 \pm 1.6 ^a

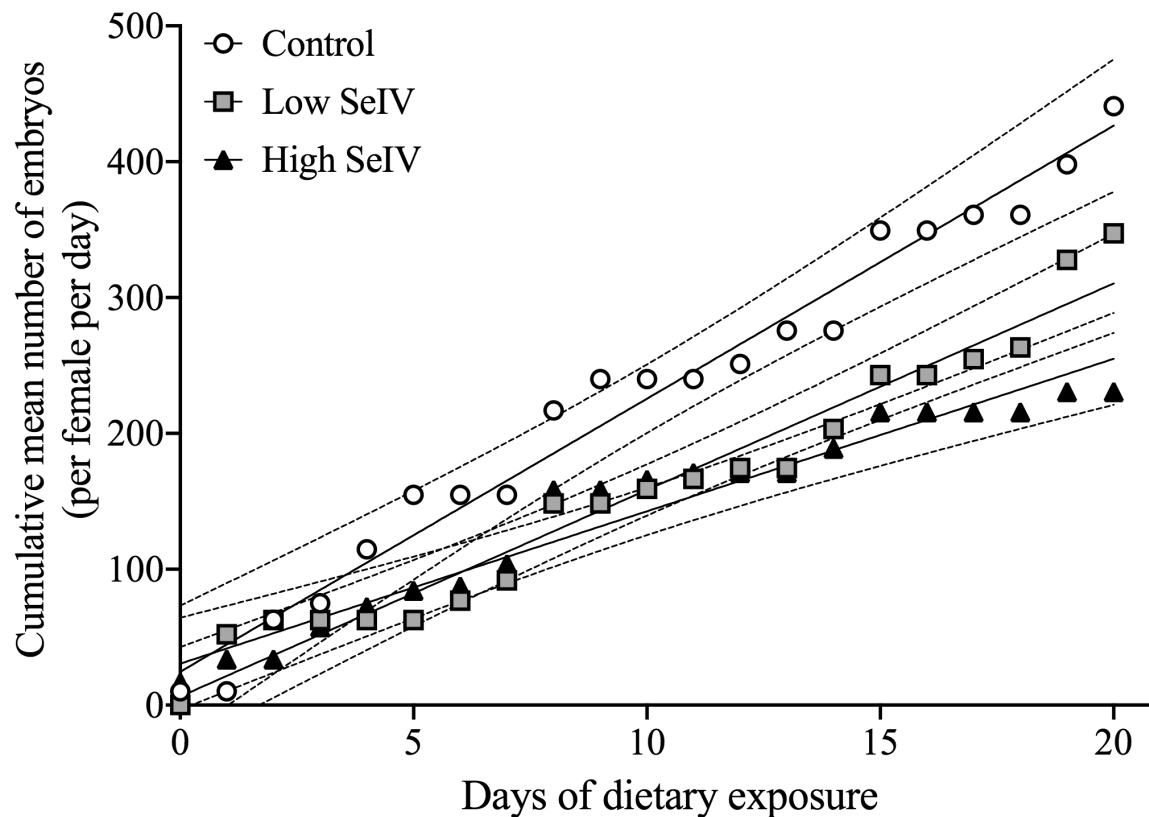


Figure 3.4. Cumulative number of mean embryos produced by *P. promelas* breeding groups during 21 days of dietary exposure to Se. The three treatment groups were Control (white circle) [$1.6 \mu\text{g Se g}^{-1} \text{ dw}$], Low SeIV (grey square) [$6.9 \mu\text{g Se g}^{-1} \text{ dw}$], and High SeIV (black triangle) [$19 \mu\text{g Se g}^{-1} \text{ dw}$]. An asterisk (*) represents a significant difference in ($p < 0.05$) in comparison to the control group. The lines represent the linear best fits of the data. Dashed lines represent the 95% confident intervals.

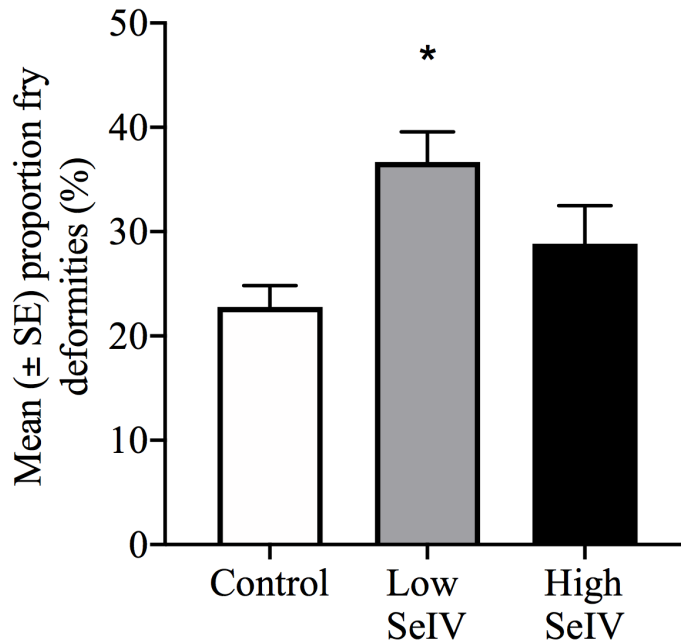


Figure 3.5. Mean (\pm SE) proportion total deformities present at swim up in F1 *P. promelas* fry fertilized by parental generation exposed to dietary Se. The three treatment groups were Control (white), Low SeIV (grey), and High SeIV (black). An asterisk (*) represents a significant difference ($p < 0.05$) in comparison to the control group.

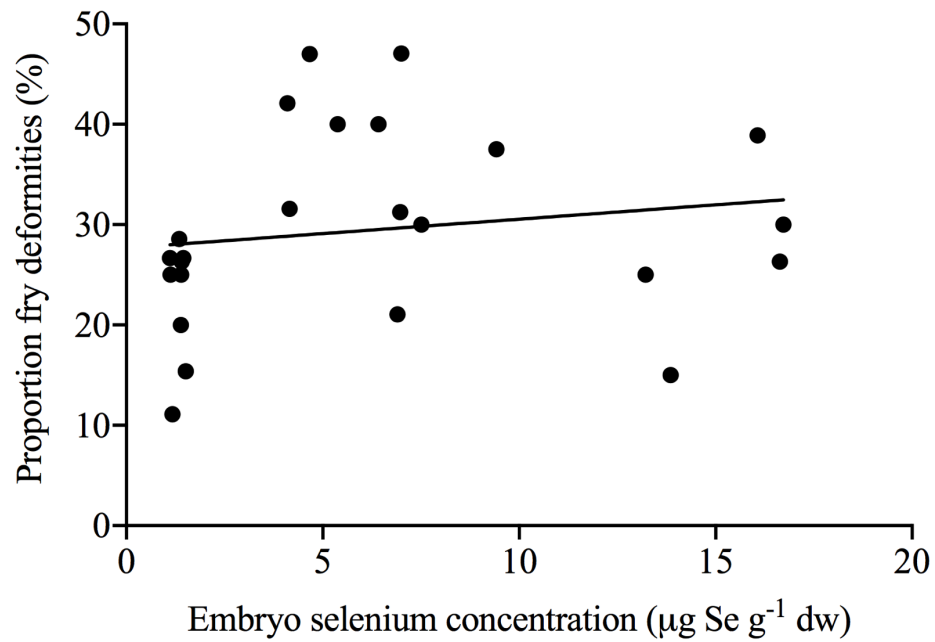


Figure 3.6. Total proportion deformities (%) observed in *P. promelas* fry relative to respective measured embryo Se concentrations (µg Se g⁻¹ dw). The line represents the linear best fit of the data.

3.5 Discussion

The present study examined the compartmentalization of Se into the tissues of a secondary consumer fish species and maternal transfer into the offspring over time. A simple laboratory-based food chain exposure was established involving a dissolved inorganic Se species previously reported as an anthropogenic contaminant. Initial aqueous concentrations of SeIV used to expose the base of the food chain in the present study were well above the BC MoE (2014) and US EPA (lentic) (2016) recommended Se ambient water quality guidelines for the protection of aquatic life (2 and 1.5 $\mu\text{g Se L}^{-1}$, respectively). The present Se exposure concentrations resulted in adverse effects on reproduction in fathead minnows within weeks of exposure. Reproductive impairment was observed in the elevated Se treatments, measured as reduced reproductive output. Observed Se maternal transfer efficiencies were less than reported by a previous study that utilized artificial dosing with SeMet-spiked diets (Lane et al. 2019), where the mean concentration of Se in embryos at steady state was up to 27 % greater than the parental diet. Mean Se concentration in the present study were 3 % and 19 % less in the low SeIV and high SeIV embryos, respectively, than the corresponding parental diets. This result is important considering that artificial Se-spiking has been the most commonly applied methodology to assess embryo toxicity of maternally transferred SeMet in fish.

3.5.1 Diet collection

Three distinct dietary Se treatments were successfully generated for the subsequent analysis of uptake and distribution of Se into the tissues and offspring of a secondary consumer fish species. However, an accurate analysis of Se trophic transfer from the exposed monoculture primary producer into the primary consumer could not be performed due to the accumulation of a periphytic-based biofilm within all the diet collection containers, which undoubtedly influenced

the Se accumulation in this portion of the food chain. Due to the consistent aqueous SeIV exposure to these two trophic levels, tissue Se residues in the primary producer and primary consumer can still offer information regarding exposure to dissolved SeIV and its trophic transfer. A trend of concentration-dependent reductions in amphipod biomass with elevated SeIV suggested chronic toxicity occurring in treatments as low as 9 $\mu\text{g Se L}^{-1}$ in *H. azteca*. This result is consistent with observations of reduced chironomid and gammarid biomass when exposed to 8.9 $\mu\text{g SeIV L}^{-1}$ in boreal lake mesocosms (Graves et al. 2019a). The extended period of Se exposure to the primary consumers also resulted in a noteworthy slowing, but continued accumulation into their tissues, suggesting the potential for an increased dietary Se exposure to secondary consumers within a system of maintained aqueous SeIV exposure.

In comparison to a previous study where green alga, *Raphidocelis subcapitata* (then *Selenastrum capricornutum*), was exposed to elevated SeIV (10 and 40 $\mu\text{g Se L}^{-1}$) then fed to larval invertebrate *Chironomus decorus* (Malchow et al. 1995), Se enrichment into the primary producer was greater despite a considerably shorter exposure period, with enrichment factors (EF) double those of the present study. Trophic transfer into the primary consumer, however, was grossly dissimilar between these studies. Where their midge larvae saw TTFs of 1.4 and 0.9 in the 10 and 40 $\mu\text{g Se L}^{-1}$ treatment groups, respectively, after 96 hours, the present study had much greater TTFs of 5.1, 5.1, and 3.8 after 4 weeks in *H. azteca* fed algae containing 1.3, 2.6, and 5.8 $\mu\text{g Se g}^{-1}$, respectively. Species-specific differences in primary producer Se enrichment are not uncommon. However, those measured in the present study were within a similar range to those of other chlorophyte species (Baines & Fisher 2001). Comparably, the higher calculated TTFs in the present study are generally above the range of previously reported transfer factors for non-filter feeding invertebrates (Andrahennadi et al. 2007; Presser & Luoma 2010), highlighting the

uncertainty caused by the unmeasured periphytic accumulation. The contribution of dissolved SeIV to the whole-body Se of the amphipods in the present study may have also been a meaningful factor, in comparison to the referenced study where dissolved Se was not maintained during the midge exposures (Malchow et al. 1995).

3.5.2 Fish tissue selenium residues

All fathead minnow tissues evaluated showed a concentration-dependent increase in Se concentration throughout respective time points. Adult livers accumulated the greatest concentrations of Se among the tissues measured, closely followed by the gonads. Female gonads showed greater Se levels than in males. Adult muscle showed the least uptake among tissues, and displayed the opposite sex relationship, with males being the greater Se accumulator. The difference between sexes here may have been a result of Se offloading through maternal transfer by the females, whereas the males would require a tissue for Se storage due to a limited rate of excretion. Fathead minnow embryos displayed a concentration-dependent relationship in their measured tissue Se concentrations, with greater Se accumulation as time progressed in embryos produced from parents exposed to elevated dietary Se.

Reproduction studies involving *P. promelas* exposures to elevated dietary Se by Ogle and Knight (1989) and Lane et al. (2019) can serve as comparison with the present study. The former study used a diet mix that was artificially dosed with a mixture of inorganic and organic Se species (25% SeMet, 25% SeVI, and 50% SeIV), producing 10 and 30 $\mu\text{g Se g}^{-1}$ dw treatments. Bioaccumulation and growth were analyzed over 98 days of exposure, after which spawning pairs were identified and a 30-day spawning period followed the first indication of spawning for each pair. At termination of experimentation, measured muscle Se concentrations (10 $\mu\text{g Se g}^{-1}$: female = 5.30 ± 1.98 , male = 4.31 ± 0.86 $\mu\text{g Se g}^{-1}$; 30 $\mu\text{g Se g}^{-1}$: female = 7.84 ± 1.41 , male = 8.77 ± 1.22

$\mu\text{g Se g}^{-1}$) were nearly double those measured in the present study. This difference was likely due to the much longer exposure period used in their experiment, suggesting that muscle tissues may not have reached steady state by termination of the present study. However, despite the difference in exposure periods, final measured gonad Se concentrations were comparable between the $10 \mu\text{g Se g}^{-1}$ treatment in the referenced study (female = 7.59 ± 1.82 , male = $5.66 \pm 2.39 \mu\text{g Se g}^{-1}$) and the $6.9 \mu\text{g Se g}^{-1}$ treatment in the present study, and gonad Se concentrations were lower in their $30 \mu\text{g Se g}^{-1}$ treatment (female = 10.92 ± 1.62 , male = $7.82 \pm 1.10 \mu\text{g Se g}^{-1}$) compared to the $19 \mu\text{g Se g}^{-1}$ treatment in the present study. This result would suggest measurable differences between results from feeding studies where exposure involved primarily inorganic Se-dosed diets compared to those produced by natural biotransformation. It could be the product of reduced uptake of the primarily inorganic Se species by the gut of the fish as opposed to organo-Se species that would dominate in a natural food chain (Janz et al. 2014). The study by Lane et al. (2019) can offer contrast to observations within fathead minnow progeny. Four treatments containing artificially SeMet-dosed diets (1.18 , 3.88 , 8.75 , and $29.6 \mu\text{g Se g}^{-1} \text{ dw}$) were fed to reproductive fathead minnows for 28 days after an initial baseline period. That study reported Se accumulation by fish embryos after 14 days ranging between 127 % and 107 % of the Se concentrations in the elevated SeMet parental feed, maternal transfer efficiency having decreased with increasing dietary Se exposures (Lane et al. 2019). In comparison, Se in fish embryos produced after 14 days of exposure in the present study reached $97 \pm 4 \%$ of the Se concentration in the parental feed in the low SeIV treatment and $81 \pm 5 \%$ of the dietary Se concentration in the parental feed in the high SeIV treatment. Differences in Se maternal transfer efficiencies between studies were slight and may suggest some disparity in the biokinetics and bioavailability of Se in diets containing artificially-dosed SeMet compared to diets containing trophically-derived Se.

3.5.3 Biological effects in fish

Generally, adult fathead minnow morphology (i.e, mass, condition, etc.) did not display any significant adverse effects from exposure to elevated dietary Se. Slight upward trends were observed in the mass and condition of adult fish exposed to the low Se dietary concentration, in contrast to a decreasing trend in the mass of the adults exposed to the high Se concentration. Adverse effects began to emerge in the progeny of Se exposed adults, observed as an increase in the total deformities observed in low Se concentration larvae, and reduced total embryo production from exposure to the high Se concentration. The total number of embryos produced displayed a trend indicative of an impact on reproduction with elevated concentrations of Se.

Total embryos produced by females exposed to elevated dietary Se were reduced compared to controls, up to a 52 % reduction observed in the high SeIV treatment. Though total observed deformities among *P. promelas* fry showed the greatest incidence in the low SeIV treatment, no correlation was detected with regard to proportion of total deformities across measured tissue Se concentrations. The former result is consistent with results from studies where semi-natural ecosystems were dosed with comparable concentrations of SeIV (10 and 30 $\mu\text{g SeIV L}^{-1}$), which produced a higher incidence of deformities in progeny of stocked fathead minnows in the lower Se treatment (Schultz & Hermanutz 1990). Similar to the present study where reproductive output was reduced in the high SeIV treatment group, somewhat hindering examination of fry deformities, their high dose produced a replicate with high standing stock mortalities that impaired their deformities analysis (Hermanutz 1992). Despite the year-long length of their exposure period, both the mean ovary and embryo Se residues were greater in the present study. However, comparisons that can be drawn between studies are limited due to differences in exposure conditions and endpoints. The aforementioned Ogle and Knight (1989) study also found no discernible effects of

elevated dietary Se on reproduction and larval success in *P. promelas*, though the mean number of embryos produced per spawn was greatest in their 10 µg Se g⁻¹ treatment, compared to all other treatments. Maternal transfer of Se into the embryos was not analyzed, and therefore could not be compared.

Despite the differences in measured embryo Se concentrations, the low SeIV concentration in the present study resulted in greater relative total deformities in comparison to the control than the comparable treatment groups in the Lane et al. (2019) study, and there was no observed adverse effect on reproductive output in their elevated Se concentrations. Conversely, their highest Se treatment group saw the greatest proportion of fry deformities. These differences would indicate an important contrast to be made regarding the method of Se-dosing for the purpose of trophic and maternal transfer analysis. The present study would suggest that parental exposure to naturally transformed Se species may result in a reduced Se bioavailability into the vitellogenin-producing hepatocytes of fish and adverse effects on reproductive output, in comparison to artificial dosing with a representative organo-Se species. Such differences have been previously speculated (Rigby et al. 2014) and still deserve further examination. Uncertainty remains regarding relative teratotoxicity caused by respective dosing methods, as reproductive disruption in the present study prevented an accurate comparison of fry deformities.

3.5.4 Conclusion

Slight differences were measured in Se bioavailability under the present trophically-derived Se exposure scenarios relative to artificial dosing with an organo-Se species. This result will be a valuable consideration for future revisions of tissue Se guidelines in aquatic life. A commonly implemented experimental exposure technique of artificially dosing feed with SeMet assumes that this organo-Se species dominates the tissues of Se-exposed, lower trophic level

organisms, but this assumption has been questioned (Rigby et al. 2014). The assumption has been supported by attempts at differentiating Se species within trophically-exposed organisms, where the selenide class of Se species, which includes SeMet, has dominated consumer tissues (Andrahennadi et al. 2007; Franz et al. 2011; Tse et al. 2012). Through the examination of Se exposure through trophically-derived diets, the present study suggests that artificial dosing with organo-Se (e.g. SeMet) may slightly overestimate the concentration received by fish embryos and underestimate adverse effects on reproductive output, though an investigation of relative teratotoxicity was indeterminate due to differences in reproductive effects. With the growth in our understanding of the trophic and maternal transfer of teratogenically-dangerous chemicals such as Se, further studies examining the relative bioavailability of naturally-dosed diets compared to those of artificial-dosing are needed. Accurate models of Se maternal transfer and its impact on embryo development are important for the reinforcement of established tissue residue guidelines for the protection of aquatic life.

CHAPTER 4

GENERAL DISCUSSION

4.1 Project rationale and research objectives

It has become increasingly clear that certain anthropogenic operations can potentially release concentrations of Se that pose a risk to ecosystems (Lemly 2004), garnering a growing list of examples where elevated concentrations have caused adverse effects in fishes (Cumbie and Van Horn 1978; Crutchfield 2000), birds (Ohlendorf 1989; Skorupa 1998), and amphibians (Hopkins et al. 2000; Punshon et al. 2005). Studies conducted in cold freshwater ecosystems representative of northern conditions have been limited. Regional-specific examination has proven fundamental in setting Se thresholds due to inherent difficulty in predicting associated environmental risk based on a single generic guideline, site-specific assessments having been proposed as an effective tool for predicting the bioaccumulation and ecological risk posed by Se contamination (McDonald & Chapman 2007).

The present research was undertaken as a portion of a larger collaborative effort to improve the ecological risk assessment of Se, particularly in cold, freshwater environments. Overall objectives of the present collaborative effort focus on satisfying knowledge gaps existing in the scientific literature. These included: 1) determination of the relative importance of community composition at the primary producer trophic level on bioconcentration (Markwart et al. 2019; this study) and trophic transfer of Se to higher organisms (this study), 2) tissue compartmentalization

of SeMet through dietary exposure into a representative cold-water fish species (this study), 3) comparison of adverse teratogenic effects from maternal transfer to a microinjected route (Lane et al. 2019), 4) -omic effects in representative fishes caused by Se exposure (Green et al., in preparation), and 5) exposure through a field-based, boreal ecosystem for an *in situ* assessment of bioaccumulation and trophic transfer (Graves et al. 2019a, 2019b).

4.1.1 Primary producer to primary consumer bioaccumulation

Results from the monoculture alga experiment found higher amphipod tissue Se concentrations with dietary and concurrent aqueous exposure. Particularly relevant to the methodology of other presented studies, most treatments showed no evidence of reaching a steady state at the time of experiment termination. These results suggest the need for longer exposure periods for the model invertebrate to reach steady state. In comparison, naturally-occurring biofilms enriched aqueous Se to a greater extent than a monoculture of green alga. Three of the biofilms tended toward the same average SeIV uptake with an overall mean of $45 \pm 3.4 \mu\text{g Se g}^{-1} \text{ dw}$ measured at the termination of experimentation in the high treatment ($25 \mu\text{g Se L}^{-1}$), while Summit Lake samples reached concentrations half of those, showing mean SeIV uptake of $22 \pm 1.3 \mu\text{g Se g}^{-1} \text{ dw}$ at the termination of experimentation. The reason for this discrepancy was likely due to significantly greater biomass collected from Summit Lake, resulting in a reduced surface area within the biofilms of these samples. Therefore, despite differences observed in the algal compositions of collected biofilms, SeIV uptake did not appear to differ as a function of community composition among biofilms, but rather as a function of primary producer abundance.

Trophic transfer of Se into the tissues of the primary consumer *H. azteca* proved less predictable in comparison to uptake by the biofilms. In the low SeIV treatment, trophic transfer factors were consistent among three sites, whereas those produced by the higher SeIV

concentration were more variable. Roadside Lake amphipods reached the greatest TTF at 0.50 ± 0.14 invertebrate:algae tissues in the high SeIV treatment group. Chris' Pond and Summit Lake amphipods reached mean ratios just over half of that from Roadside Lake at 0.25 ± 0.03 and 0.26 ± 0.07 invertebrate:algae tissues, and Smeaton Pond showed an even further reduced mean ratio at 0.15 ± 0.03 invertebrate:algae tissues. The observed reductions in trophic transfer measured in Smeaton Pond samples are perhaps the most easily explicable, owing to an influence of algal community composition where cyanophytes were most abundant in this site's biofilms. The assimilation efficiency of cyanophytes into the diet of *H. azteca* is significantly reduced in comparison to other bacteria, diatoms and green algae microorganism species (Hargrave 1970), rendering feeding avoidance a significant influencing factor in the ultimate uptake of Se into the present model invertebrate. High iron oxide concentrations were also measured in the Smeaton Pond biofilms in Markwart et al. (2019), which could have also resulted in avoidance by the amphipods. Where trophic transfer into Chris' Pond invertebrates in the high SeIV treatment was reduced in comparison to Roadside Lake despite comparable mean biofilm Se tissue residues, the similarity to results from Summit Lake may shed some light on the reason. A mean of tissue Se measurements taken at the beginning and termination of experimentation does not necessarily account for differences in the time to reach steady state among different biofilms. Whereas Roadside Lake biofilms had reached Se concentrations at the beginning of the feeding exposure comparable to those at its termination, Chris' Pond biofilms were continuing to accumulate Se when the feeding exposure commenced. The relative influence of equal sampling at the beginning and termination of experimentation may have biased mean biofilm Se concentrations measured in Chris' Pond samples toward the higher final tissue concentrations. This hypothesis is corroborated

by the comparable trophic transfer factors measured in Summit Lake samples, where accumulation of Se continued throughout the feeding exposure.

In contrast to results measured for the SeIV chemical species, uptake of SeVI was negligible in all biofilms regardless of treatment concentration. This result is consistent with previous studies where high aqueous sulfate (SO_4) concentrations reduce SeVI bioavailability at the base of the food web due to competition for uptake (Williams et al. 1994; Lo et al. 2015). Test water in the present study was sourced from Saskatoon municipal water, with SO_4 ion concentrations measured at $86 \pm 6.1 \text{ mg SO}_4 \text{ L}^{-1}$. Measured SO_4 concentrations were in the range of SeVI screening guidelines recommended by DeForest et al. (2017) of $14\text{--}21 \text{ }\mu\text{g SeVI L}^{-1}$ when background SO_4 concentrations occur between $75\text{--}100 \text{ mg SO}_4 \text{ L}^{-1}$. The present study generally agreed with the protective nature of these recommended guidelines under the presented conditions, where Se was not significantly bioconcentrated by the biofilms at concentrations as high as $25 \text{ }\mu\text{g SeVI L}^{-1}$ and therefore not rendered bioavailable to higher trophic levels, with the exception of one site showing reduced relative growth in the invertebrates and higher Se residues. Due to the negligible uptake of the SeVI chemical species measured in the aforementioned test conditions, SeIV was chosen for the focus of the laboratory food chain exposure into the secondary consumer fish species.

4.1.2 Primary consumer to secondary consumer bioaccumulation

Fundamental to the collection of consistent Se concentrations in the tissue residues of the fish feed, the latter had to be fed a constant Se diet themselves and time to reach steady state was a particular consideration. *H. azteca* were housed in environmental conditions with concurrent aqueous exposure to dissolved SeIV concentrations identical to that exposed to their feed, green alga *S. bacillarus*. The need of six weeks for the amphipods in the two highest SeIV treatments to

reach tissue Se steady state suggests that Se exposure concentration is a factor in the time necessary to reach steady state, with greater concentrations requiring longer to reach maximum tissue residues. Measured tissue residues in the two highest SeIV treatments also seemed to reach comparable levels, showing mean Se tissue residues of $26 \pm 2.7 \mu\text{g Se g}^{-1}$ after 10 weeks of exposure in the medium treatment, relative to the mean of $31 \pm 3.1 \mu\text{g Se g}^{-1}$ in the high treatment throughout exposure. Chronic adverse effects became apparent in the highest SeIV, where available invertebrate biomass was reduced relative to the lower concentrations, anecdotally suggesting chronic toxicity of SeIV in invertebrates. Based on the reduction in available *H. azteca* collected from the high SeIV treatment and similar tissue Se residues reached in the medium after 10 weeks of diet collection, these two invertebrate treatments were amalgamated into a single fish food treatment, with a mean tissue Se concentration of $29 \pm 2.3 \mu\text{g Se g}^{-1} \text{ dw}$. The control and low Se amphipod treatments measured mean tissue Se concentrations at 1.9 ± 0.5 and $10 \pm 1.3 \mu\text{g Se g}^{-1} \text{ dw}$ respectively.

A fathead minnow reproductive assay was performed following the sufficient collection of what would become their selenized feed. Se uptake into the tissues of adult *Pimephales promelas* and their embryos displayed a concentration-dependent relationship, greater uptake measured with elevated exposure concentrations. The greatest tissue Se concentration among all life stages and treatments was measured in embryos produced by the high treatment after fourteen days of exposure, analyzed at $17 \mu\text{g Se g}^{-1} \text{ dw}$. Mean Se in the female gonads at the termination of experimentation were comparable in this treatment, measuring $16 \pm 1.8 \mu\text{g Se g}^{-1} \text{ dw}$; however, the livers showed greater Se accumulation in the adult fish, measuring 17 ± 1.9 and $17 \pm 1.3 \mu\text{g Se g}^{-1} \text{ dw}$ in the females and males, respectively. Hatchling deformities were statistically greater

in the low SeIV treatment groups, but reduced embryo production in the high treatment resulted in an inadequate sample size to properly assess teratogenic effects.

4.1.3 Integration of results

At the level of the primary producer, Se tissue enrichment could be compared across experiments after eight days of exposure. At the SeIV treatment concentrations in the range of 25-27 $\mu\text{g Se L}^{-1}$, Se tissue residues were highly inconsistent between experiments. Where the monoculture green algae tissues reached a mean concentration of $2.6 \pm 0.4 \mu\text{g Se g}^{-1} \text{ dw}$ for the minnow feeding study, biofilm samples were at a minimum 6.6- to 17.5-fold greater than those of the monoculture among the high SeIV exposure treatment groups. Differences in SeIV enrichment produced by the experimental conditions could theoretically be explained by the differences in composition of the primary producers, and the chemistries of utilized test waters. A source of uncertainty was produced by the nature of the complex composition of the naturally-collected biofilms. Whereas the tested monoculture was composed of a single green alga species, biofilms were by definition matrices of microorganisms. Differences in Se enrichment by these various taxa have been poorly studied. However, perhaps the largest source of variability between experimental enrichment can be explained by difference in water chemistries, particularly in the concentrations of phosphate ions (PO_4^{3-}) present in the test systems. Previous research has found reduced SeIV uptake by green alga *Chlamydomonas reinhardtii* with increasing PO_4^{3-} concentrations (Riedel & Sanders 1996). Bold's Basal Media, a high nutrient water recipe used for the growth of the monoculture green algae, contains $160 \text{ mg PO}_4 \text{ L}^{-1}$, in comparison to the negligible concentrations present in Saskatoon municipal water, the test water utilized for the biofilm exposure.

Naturally-collected biofilm results can be further compared to work by Markwart et al. (2019), which used identical sampling and biofilm housing methods but under different water

chemistry conditions. In this experiment, test water followed an Environment and Climate Change Canada formulation, where test water ion concentrations were greatly reduced in comparison to the Saskatoon Municipal water utilized in the present biofilm study. Se tissue residues measured in Markwart et al. (2019) were generally elevated in comparison to the present research after eight days of exposure. Summit Lake and Chris' Pond biofilms reached the most similar concentrations between studies, where their biofilms were 1.4-fold and 1.5-fold greater in the 25 $\mu\text{g Se L}^{-1}$ treatments, respectively, than concentrations measured here. Smeaton Pond showed the most dramatic difference, their biofilms reaching concentrations 4.8-fold greater than the present study. The dramatic difference in uptake observed in their Smeaton Pond samples was hypothesized to be a result of the high affinity of SeIV for iron oxyhydroxides (Balistreiri and Chao 1990), the latter being present in considerably higher concentrations in the biofilms collected at this site. The lack of difference in measured SeIV concentrations in the biofilms of the present study is curious, but test water chemistries are most likely responsible. PO_4 , silicate, and molybdate have been found to compete and decrease the adsorption of SeIV to iron oxyhydroxides (Balistreiri and Chao 1990). Though the concentrations of these known interacting ions do not significantly differ between experiments, being present in both cases at comparably negligible concentrations, the presence of other ions at elevated concentrations may explain the differences in SeIV enrichment measured in the Smeaton Pond biofilms. The adsorption of trace metals (e.g. Cd, Cu, Ni, Pb, Zn) onto natural iron oxyhydroxides have been reported in the literature (Tessier et al. 1985), and strongly binding anionic adsorbates (e.g. AsO_4^{3-} , AsO_3^{3-} , CrO_4^{2-} , PO_4^{3-}) have been found to have minor interaction with such metal ions (Benjamin & Bloom 1981). Difference in test water pH between studies within a range that has demonstrated pH-dependent adsorption of SeIV to iron oxyhydroxides was also a likely influence (Balistrieri & Chao 1990). Large differences in test

water compositions were therefore theoretically responsible for the differences in measured SeIV adsorption, and thus, apparent biofilm Se enrichment measured in Smeaton Pond samples, where elevated ion concentrations (e.g. HCO_3^- , SO_4^{2-}) and pH in the present study influenced Se oxyanions adsorption to iron oxyhydroxides, contributing to a reduction in measured Se within the biofilms.

The role of Se adsorption to inorganic constituents within biofilms highlighted by these studies further develop the question of bioavailability to higher consumer species. High concentrations of iron measured in biofilms collected from Smeaton Pond were most likely associated with the extracellular environment (Letovsky et al. 2012). Due to similarity in SeIV accumulation in natural biofilms with accumulation in heat-killed biofilms, Markwart et al. (2019) concluded that the apparent Se enrichment measured in iron-elevated Smeaton Pond biofilms were a result of adsorbent-adsorbate interactions rather than active biological uptake. In general, organic Se species that are the result of biotransformation after absorption into primary producer tissues are more bioavailable to primary consumers than the original inorganic Se species (Simmons & Wallschläger 2005). Therefore, inorganic Se extracellularly adsorbed within biofilm matrices would be less bioavailable in comparison to absorbed and biotransformed Se species at similar total Se concentrations. For this reason, despite significant differences in total Se measured in Smeaton Pond biofilms between experiments, the presence of high levels of iron within the systems should be considered as a contributing factor to the comparably low uptake of Se into the primary consumer tissues measured in the present study. However, the relative contribution of reduced Se bioavailability in the trophic transfer of Se to consumers compared to feeding inhibition caused by the high occurrence of cyanophytes remains inconclusive within this experimental design.

When considering the results of Se uptake into the primary consumer in relation to measured uptake into fish species, uncertainties should be fewer, as transfer between these trophic levels have been better characterized, showing much less variability (Luoma & Presser 2009); however, ambiguity remains relating to Se speciation, selective feeding, and dosing method. Perhaps the most meaningful result from the present research was the difference in maternally transferred Se compared to another study (Lane et al. 2019), suggesting that the commonly used methodology of artificial dosing with organo-Se may overestimate the concentration received by fish progeny and underestimate that retained within the mother. This difference may be indicative of a disparity in the biokinetics and bioavailability of artificially-dosed organo-Se feed relative to exposure to trophically-derived Se concentrations. This conclusion is particularly noteworthy given the use of differing Se dosing methods for setting regulatory guidelines.

4.2 Recommendations

Throughout this research project, numerous themes emerged where improvements could be made or further research could enhance understanding regarding the biogeochemical factors involved in the enrichment and trophic transfer of inorganic Se chemical species within freshwater ecosystems. The primary purpose of the present research was to address knowledge gaps existing in the Se literature regarding influence at the base of the food web on the enrichment of the most common anthropogenically released inorganic Se species, and the trophic transfer of naturally-derived sources of Se-dosed feed, originating from the same aqueous-borne inorganic chemical species. This research project was successful in addressing the original objectives, where 1) the influence of the algal component of biofilm communities on trophic transfer to the primary consumer trophic level was evaluated, and 2) tissue distributions and maternal transfer of Se in a fish species were measured, resulting from exposure to a laboratory grown diet, through natural

aqueous dosing and trophic transfer at base levels of the food web. The presented results assist in describing the biogeochemical relationships in Se cycling at the primary producer trophic level for further trophic transfer to more sensitive, higher organisms. However, as is common in most scientific inquiry, the results also highlighted areas where knowledge is lacking and raised additional questions for future consideration.

4.2.1 Natural biofilm community composition

Despite apparent differences in biofilm community composition, bioconcentration of Se measured in the biofilm tissues was similar among sites, aside from where surface area was a factor due to a large difference in collected biomass from one site. This result became particularly interesting when trophic transfer into the primary consumer invertebrate resulted in varying Se tissue residues among site biofilms (Chapter 2). Hypotheses that arose to explain the lowest invertebrate accumulation were derived from an influence of algal community composition, where cyanophytes were most abundantly observed, a family known to show reduced assimilation by this model invertebrate species. The question of Se bioavailability was also a consideration due to the presence of elevated iron at this field site, which is known to strongly adsorb with inorganic Se chemical species. Ultimately, the reduced accumulation of Se within this particular system was likely a result of both of the aforementioned factors; however, additional questions emerged with these results in mind.

An issue that continues to pervade the assumptions made in biofilm research regards the taxonomic composition of these biofilms, the relative influence of different biologic kingdoms, and potential feeding preferences of the primary consumers. For future consideration, as databases for lower taxonomic rankings become more widely available, a comparison between the molecular composition of the biofilm and that within the gut of the consumers, using emerging eDNA

methodology, would be a relatively straightforward method to elucidate any selective feeding that may be occurring. Differential exposures to a range of primary producer kingdoms would also be important to determine any relative difference in Se uptake that may be occurring, for the interpolation of Se uptake into assemblages.

4.2.2 Biogeochemical interactions

Toxicity resulting from exposure to elevated concentrations of Se involves a host of collaborating factors. Site-specific differences in biogeochemical constituents have been greatly implicated in resulting disparities in the bioaccumulation and trophic transfer of Se species in aquatic ecosystems (Lemly 1999). Concentrations of SO_4 , PO_4 , and iron are among the chemical components that have been previously identified as having influence over the ultimate uptake of Se into a system (Riedel & Sanders 1996; Markwart et al. 2019), rendering attempts at generalized modeling of Se trophic transfer difficult. Numerous additional sources of Se interaction that require further investigation likely exist due to the occurrence of chemical mixtures resulting from anthropogenic activities. Analysis of potential sources of Se interactions with other geochemical components should be a fundamental consideration for future research, particularly in the case of factors that are well established as influencing Se uptake. A well-developed plan for risk assessment and management of Se-impacted ecosystems requires a thorough weight-of-evidence approach, where findings from numerous studies are compiled to determine the most common trends, an approach that would greatly benefit from the consistent reporting of known interacting factors, such as SO_4 and PO_4 , for a greater comprehension of outcomes involving variable competing factors.

4.2.3 Selenium maternal transfer

The present study was successful in simulating the assimilation of Se into the tissues of reproductively active fish and their offspring, through a naturally-derived laboratory food chain. Teratogenic effects and a trend toward reduced reproductive output observed in the low and high SeIV fish exposure treatments, respectively, were generally consistent with previous studies, and represented the attainment of controlled, representative Se bioconcentration and trophic transfer within realistic conditions. However, uncertainties were encountered that limited the efficacy of teratogenic endpoints. Due to reduced reproduction in the high SeIV concentration, proper replication could not be attained to assess deformities across collection periods within this treatment group. Though the result of reduced reproduction is consistent with previous studies, a better understanding of the Se concentration-response in fish progeny could be obtained through multiple experimental design adjustments. Firstly, further parental aquaria replicates could be added to maximize the potential of adequate replication of progeny, though this would not guarantee sufficient numbers. Additionally, more treatment concentrations could be added to better characterize the relationship between maternally-transferred Se in progeny and resulting deformities.

Regarding the objective of tracking trophic transfer from the dissolved phase through the entirety of the constructed food chain, certain TTFs remained inconclusive due to uncharacterized Se accumulation in the periphytic build up within amphipod collection microcosms. Trophic transfer efficiencies resulting from the initial aqueous concentrations of SeIV exposed to these systems rendered comparison of Se concentrations between primary producer and primary consumer trophic levels ineffective. Sampling of this periphytic build up would have established more accurate TTFs for a thorough determination of trophic dynamics. Furthermore, a commonly

cited explanation for the lack of such experimental design, involving Se transference from the dissolved phase through higher consumer trophic levels, has been the necessity for considerable time and resources for its completion. These obstacles definitely occurred in the present study, relating to the collection of sufficient Se-dosed diets to satisfy the needs of the reproductive assay. Therefore, it seems appropriate that such factors should be considered in the pursuit of similar experimental designs, and should be approached with significant consideration and contingencies.

4.3 Future research opportunities

Although the present research contributes to our understanding of Se uptake and trophic transfer, important additional avenues for further future scientific exploration remain. Given the multitude of factors affecting the bioconcentration and trophic transfer of Se species, there is no shortage of parameters that require characterization and potential further exploration. For example, interactions caused by SO_4 affecting the uptake and trophic transfer of SeVI have been thoroughly examined (Hansen et al. 1993; Williams et al. 1994), and are also further supported by the results of the present research. In comparison, interactions in the uptake of SeIV by PO_4 have been previously reported (Riedel & Sanders 1996); however, a detailed investigation of this relationship remains outstanding. Furthermore, a compilation of these and other influencing relationships could be utilized to bolster existing biokinetic Se uptake models (DeForest et al. 2015), including consideration for other factors such as nitrate, for its interaction potential but also due to its role in primary producer growth. Given the highly variable enrichment of Se by the primary producer trophic step, and its foremost impact on exposure to higher, more sensitive trophic levels, the thorough characterization of influencing factors at this base level is fundamental for the protection of the greater ecosystem. Relating observed site-specific characteristics to Se uptake and trophic transfer to species of greatest concern in impacted or potential future receiving aquatic

environments would be valuable for establishing appropriate Se exposure limits for the protection of distinct environments. Other future research opportunities more directly recognized through the present research include the need for a more thorough examination of Se bioconcentration by the highly diverse components that comprise naturally occurring biofilms, as well as the investigation of naturally-derived Se trophic transfer to a greater diversity of invertebrate and vertebrate species, with a focus on aqueous origins that are most representative of encountered chemical species and concentrations.

4.3.1 Selenium bioconcentration by natural biofilm components

Research dedicated to the investigation of Se uptake into naturally-occurring biofilms has been an important area of focus in recent years (Muscatello et al. 2008; Conley et al. 2009; Scheibener et al. 2017; Markwart et al. 2019), especially given the fundamental importance of the primary producer trophic level in the bioconcentration of aqueous Se and its position as a dietary pathway for trophic transfer to higher level organisms in periphyton-based aquatic food webs. Though biofilms represent an important food source, knowledge gaps persist regarding relative Se bioconcentration by their different taxonomic components, which may influence the trophic transfer efficiency observed. Selective feeding, a phenomenon where grazers will preferentially consume a preferred subset of available fodder, has been observed in aquatic invertebrates. Previous research has documented invertebrate selective feeding relating to such factors as size and spatial partitioning among the grazer community (Tall et al. 2006) and taxonomic composition of available diets (Hargrave 1970), influences that can alter growth and nutrient partitioning in organisms as they go through their life cycle (Baker & McLachlan 1979).

Due to the inherent variability in the composition of periphytic communities, which can include algae, bacteria, fungi, exopolymeric substances, small eukaryotes, and other particulate

matter, an understanding of the relationship between these components and the potential uptake of Se into consumer trophic species is necessary for a more comprehensive sense of trophic transfer efficiencies within natural ecosystems. Research has begun to assess differences in Se bioconcentration as a function of isolated, monoculture primary producer species, namely in diatoms, chlorophytes, and dinoflagellates, etc., having reported variable Se enrichment among taxa (Harrison et al. 1988; Baines & Fisher 2001; Lo et al. 2015). However, an examination of the relative bioconcentration of Se in such components isolated from naturally-occurring periphyton composites and any feeding preference expressed by consumer trophic species may alter the formulation of biokinetic models for Se bioconcentration and trophic transfer. Though this experimentation would be highly valuable, a presumable reason for its lack of progress is likely the question of methodology. A lack of information exists in the literature related to the isolation of periphytic components; therefore, such an examination would necessitate the development and experimentation of protocols. One component that has been previously isolated from periphyton that can offer initial recommendations are the exopolymeric substances. The composition of this constituent from periphytic assemblages has been a focus of phycological research, as significant effects of saccharide composition among varying periphytic assemblages on biogeochemical processes have been proposed (Browder et al. 1994; Gaiser et al. 2006; Bellinger et al. 2010). The extraction of the exopolymeric substances in these examples involves multiple centrifugation and supernatant isolation steps, methods that may be used as a starting point for the development of appropriate component isolation protocols. Another aspect that requires further investigation is the potential exposure of early life stage aquatic vertebrates to elevated Se through their ingestion of biofilms, as the contribution of this trophic level to the diets of larval stage vertebrates has not been greatly investigated.

4.3.2 Inorganic selenium bioaccumulation in multiple invertebrate and vertebrate species

An area of Se research that requires further examination involves the comparison of dietary uptake of Se into consumers and their offspring from naturally selenized diets and those resulting from artificial dosing with SeMet. This research is important to decide whether the less resource intensive method of artificial-spiking the diet is a reasonable surrogate for the assessment of Se biokinetics or alternatively to determine whether there is consistency in any observed difference in the biokinetics of naturally-dosed and artificially-spiked diets, for the determination of appropriate modeling strategies. The present research was intended to serve this comparative purpose through a dosing regimen that closely resembled that reported in Lane et al. (2019), where feed was artificially spiked with SeMet. With dietary Se at similar concentrations in the fish feed between studies, a difference in Se maternally transferred to the offspring was observed, highlighting the necessity to examine such differences in experimental design. Significant research exists relating to multiple dosing scenarios of consumer trophic levels, particularly involving the analysis of marine invertebrate (Reinfelder et al. 1997; Wang & Fisher 1996) and vertebrate species (Reinfelder & Fisher 1994; Baines et al. 2002; Wang 2002). However, research related to the complexities of Se dosing in freshwater food chains remains comparably less studied.

Studies involving the bioconcentration of inorganic Se species into primary producers, phytoplankton, and periphytic assemblages (Riedel et al. 1996; Yu & Wang 2004; Markwart et al. 2019), and trophic transfer to consumers through diets of laboratory based, naturally bioconcentrated Se (Besser et al. 1993; Conley et al. 2009), artificially-spiked Se (Misra et al. 2012; Thomas & Janz 2015; Pettem et al. 2017; Lane et al. 2019), or sourced from Se-impacted ecosystems (Andrahennadi et al. 2007; Phibbs et al. 2011; Franz et al. 2013; Janz et al. 2014) have begun to characterize Se uptake and trophic transfer in freshwater ecosystems. Despite the

significant surge in the analysis of Se in higher trophic levels of freshwater systems, a great deal of research continues to be outstanding, particularly in the form of relating results from artificial-spiking to naturally-transferred Se, site-specific biogeochemical differences, and the potential for acquired tolerance in Se-impacted ecosystems. Though the present research suggests that there are differences in Se biokinetics between dosing methods, replication of similar experimental designs is required to strengthen this conclusion.

4.4 Summary

The purpose of the present research was to assist in filling knowledge gaps that exist regarding the bioconcentration and trophic transfer of inorganic Se species through representative cold freshwater food chains. Specific objectives were to investigate the influence of primary producers on the bioconcentration and trophic transfer of Se, the primary producer trophic level having been known as the greatest Se enrichment step, but also proportionally the least understood. This was accomplished through exposure of biofilms with variable community compositions to SeIV and SeVI, and the trophic transfer to a common primary consumer species, to identify possible differences influencing Se uptake into the dietary component of higher consumers. Differences in Se residues in the primary consumers were measured among SeIV treatment groups relating to site-specific biofilms, hypothesized to have been related to reduced surface area occurring within a system composed of greater biomass or the avoidance of particularly unpalatable species through selective grazing. Comparably, enrichment of SeVI was negligible in all biofilms and amphipods regardless of treatment concentration, believed to have occurred due to aqueous SO₄ in test water, an ion known to reduce SeVI bioavailability at the base of the food web due to competition for uptake (Williams et al. 1994; Lo et al. 2015).

The secondary objective of the present research was to characterize the enrichment and trophic transfer of Se through an environmentally relevant food chain, exposure beginning through the aqueous phase as inorganic Se into primary producers and ending at the secondary consumer trophic level. The study was designed for comparison to studies that used artificially-dosed diets with an organo-Se species, because such dietary dosing has been widely used with the assumption that they comprise the greatest portion of Se within naturally-transformed systems. Simulating a naturally-transferred Se food chain was successful, having characterized resulting tissue residues in male and female fathead minnows, and their offspring. An increase in deformities was observed in the low SeIV treatment group's progeny, and reduced reproduction occurred with exposure to elevated Se treatments. Results also indicated an overestimation of maternally transferred Se in a comparable study having artificially-dosed the adult diet with SeMet (Lane et al. 2019). The present results will serve to inform biokinetic models for exposure to aqueous inorganic Se species, the chemical species of greatest concern for its release from anthropogenic activities.

The present research thesis was successful in contributing information to the existing Se literature; however, knowledge gaps continue to persist regarding the kinetics of Se within the highly variable conditions of potential receiving aquatic ecosystems. Pressure for site-specific Se thresholds based on prevailing conditions for the appropriate protection of these specific ecosystems has been gaining advocacy in recent years. Though research has begun to interpret trophic level interactions, outstanding questions particularly relating to the influence of site-specific biogeochemical parameters on Se enrichment by the primary producer trophic level require further exploration for the development of the most appropriate guideline strategy. Existing guidelines also haven't considered toxicity at trophic levels below the secondary consumer, an assumption that is beginning to lose support due to evidence supporting toxic effects of Se in

primary consumer species (Graves et al. 2019a). Current Se management approaches have been effective in limiting instances of environmental disruption. Going forward, consideration of lower trophic levels is recommended in the development of Se guidelines for the protection of the entire ecosystem.

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APPENDIX A

SUPPLEMENTARY INFORMATION FOR CHAPTERS 2 AND 3

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Field Site	Treatment	Temperature ($^{\circ}\text{C}$)	DO (mg L^{-1})	Conductivity ($\mu\text{S cm}^{-1}$)	pH	Alkalinity (mg L^{-1})	Hardness (mg L^{-1})	PAR ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Se ($\mu\text{g L}^{-1}$)
Chris' Pond	Control	20 ± 0.27	7.9 ± 0.12	467 ± 8.1	8.0 ± 0.04	132 ± 2	175 ± 3	17 ± 6	0.33 ± 0.04
	Low Se(IV)	20 ± 0.21	8.0 ± 0.15	463 ± 9.5	8.0 ± 0.03	130 ± 2	173 ± 4	16 ± 5	5.0 ± 0.29
	High Se(IV)	21 ± 0.19	8.0 ± 0.14	478 ± 6.7	8.1 ± 0.03	131 ± 1	174 ± 2	18 ± 5	24 ± 0.87
	Low Se(VI)	20 ± 0.31	8.1 ± 0.13	464 ± 11	8.1 ± 0.02	131 ± 2	171 ± 3	16 ± 4	5.1 ± 0.45
	High Se(VI)	21 ± 0.17	8.2 ± 0.15	479 ± 8.2	8.1 ± 0.02	132 ± 1	171 ± 3	15 ± 3	25 ± 1.33
Summit Lake	Control	20 ± 0.17	7.7 ± 0.27	506 ± 8.9	8.1 ± 0.04	132 ± 2	169 ± 3	16 ± 3	0.30 ± 0.09
	Low Se(IV)	20 ± 0.18	7.7 ± 0.24	502 ± 7.3	8.1 ± 0.03	134 ± 4	169 ± 5	17 ± 3	4.4 ± 0.38
	High Se(IV)	20 ± 0.13	8.4 ± 0.48	503 ± 6.0	8.1 ± 0.03	132 ± 3	172 ± 3	16 ± 4	22 ± 1.69
	Low Se(VI)	20 ± 0.17	8.2 ± 0.31	503 ± 5.2	8.1 ± 0.04	133 ± 3	173 ± 2	17 ± 6	5.0 ± 0.18
	High Se(VI)	20 ± 0.18	8.5 ± 0.48	503 ± 5.7	8.2 ± 0.09	129 ± 3	166 ± 3	18 ± 4	24 ± 0.77

Table A.2.2. Summary of mean (\pm SE) general water quality parameters, photosynthetically active light, and aqueous Se concentrations measured throughout laboratory exposures to respective field-collected biofilm and *H. azteca* treatments ($0.33 \mu\text{g Se L}^{-1}$ [control], $5 \mu\text{g SeIV/SeVI L}^{-1}$, and $25 \mu\text{g SeIV/SeVI L}^{-1}$).

Field Site	Treatment	Temperature (°C)	DO (mg L ⁻¹)	Conductivity ($\mu\text{S cm}^{-1}$)	pH	Alkalinity (mg L ⁻¹)	Hardness (mg L ⁻¹)	PAR ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Se ($\mu\text{g L}^{-1}$)
Roadside Lake	Control	21 \pm 0.44	7.2 \pm 0.14	469 \pm 9.7	8.0 \pm 0.04	140 \pm 3	184 \pm 5	16 \pm 3	0.43 \pm 0.06
	Low Se(IV)	20 \pm 0.36	7.5 \pm 0.12	477 \pm 9.5	8.1 \pm 0.04	142 \pm 3	184 \pm 5	15 \pm 3	5.3 \pm 0.29
	High Se(IV)	20 \pm 0.24	7.6 \pm 0.08	481 \pm 9.9	8.2 \pm 0.03	140 \pm 4	184 \pm 5	18 \pm 5	25 \pm 0.78
	Low Se(VI)	20 \pm 0.25	7.6 \pm 0.11	483 \pm 14	8.2 \pm 0.03	140 \pm 4	182 \pm 6	17 \pm 6	5.3 \pm 0.38
	High Se(VI)	20 \pm 0.24	7.7 \pm 0.14	488 \pm 9.9	8.2 \pm 0.03	138 \pm 5	181 \pm 7	15 \pm 3	25 \pm 1.73
Smeaton Pond	Control	20 \pm 0.15	7.7 \pm 0.10	509 \pm 5.0	8.2 \pm 0.04	136 \pm 3	175 \pm 3	16 \pm 4	0.37 \pm 0.06
	Low Se(IV)	20 \pm 0.12	7.8 \pm 0.09	510 \pm 4.5	8.3 \pm 0.03	132 \pm 2	170 \pm 3	17 \pm 3	6.0 \pm 1.34
	High Se(IV)	20 \pm 0.24	7.7 \pm 0.10	512 \pm 5.5	8.3 \pm 0.03	131 \pm 2	172 \pm 2	16 \pm 3	26 \pm 3.27
	Low Se(VI)	20 \pm 0.11	7.7 \pm 0.10	509 \pm 4.8	8.3 \pm 0.03	131 \pm 2	172 \pm 3	17 \pm 5	5.6 \pm 0.30
	High Se(VI)	20 \pm 0.12	7.8 \pm 0.10	511 \pm 6.1	8.3 \pm 0.03	132 \pm 2	172 \pm 2	17 \pm 5	29 \pm 2.27

Table A.2.3. Summary of mean (\pm SE) total biofilm Se concentrations ($\mu\text{g Se g}^{-1}$ dw) on experiment days 0 and 14, for biofilm samples collected from four field sites (Chris' Lake, Summit Lake, Roadside Lake, and Smeaton Pond), subsequently exposed to respective aqueous Se treatments ($0.33 \mu\text{g Se L}^{-1}$, $5 \mu\text{g SeIV/SeVI L}^{-1}$, and $25 \mu\text{g SeIV/SeVI L}^{-1}$).

Field Site		Treatment				
		Control	Low Se(IV)	High Se(IV)	Low Se(VI)	High Se(VI)
Chris' Pond	Day 0	1.5 ± 0.08	12 ± 0.76	33 ± 12	2.5 ± 0.44	3.7 ± 0.33
	Day 14	1.4 ± 0.08	10 ± 0.81	47 ± 4.1	2.5 ± 0.48	2.8 ± 0.43
Summit Lake	Day 0	1.4 ± 0.18	4.6 ± 0.10	12 ± 0.95	2.4 ± 0.80	3.0 ± 0.30
	Day 14	1.6 ± 0.07	8.0 ± 1.3	22 ± 1.3	3.5 ± 0.27	3.1 ± 0.37
Roadside Lake	Day 0	2.0 ± 0.26	9.3 ± 2.1	36 ± 1.6	1.9 ± 0.38	2.4 ± 0.31
	Day 14	2.1 ± 0.31	15 ± 3.7	43 ± 3.0	1.0 ± 0.48	2.6 ± 0.40
Smeaton Pond	Day 0	1.4 ± 0.16	9.8 ± 2.5	46 ± 9.5	1.8 ± 0.21	2.8 ± 0.60
	Day 14	2.2 ± 0.64	20 ± 5.8	44 ± 3.1	4.7 ± 0.25	11 ± 5.4

Table A.2.4. Mean enrichment functions of Se (\pm SE), calculated based on exposures from the aqueous phase ($0.33 \mu\text{g Se L}^{-1}$ [control], $5 \mu\text{g SeIV/SeVI L}^{-1}$, and $25 \mu\text{g SeIV/SeVI L}^{-1}$) into biofilms collected from four field sites (Chris' Pond, Summit Lake, Roadside Lake, and Smeaton Pond).

Field Site	Treatment				
	Control	Low Se(IV)	High Se(IV)	Low Se(VI)	High Se(VI)
Chris' Pond	4133 ± 150	2234 ± 143	1771 ± 244	535 ± 62	132 ± 13
Summit Lake	4607 ± 291	1477 ± 224	817 ± 110	608 ± 93	127 ± 9
Roadside Lake	4376 ± 386	2356 ± 445	1639 ± 84	274 ± 62	96 ± 9
Smeaton Pond	6029 ± 1150	2969 ± 690	1815 ± 179	644 ± 132	272 ± 120

Table A.2.5. Mean TTFs of Se (\pm SE), calculated based on final tissue Se concentrations in *H. azteca*, exposed to diets of Se-exposed biofilm diets ($0.33 \mu\text{g Se L}^{-1}$ [control], $5 \mu\text{g SeIV/SeVI L}^{-1}$, and $25 \mu\text{g SeIV/SeVI L}^{-1}$), collected from four field sites (Chris' Pond, Summit Lake, Roadside Lake, and Smeaton Pond).

Field Site	Treatment				
	Control	Low Se(IV)	High Se(IV)	Low Se(VI)	High Se(VI)
Chris' Pond	0.60 ± 0.23	0.46 ± 0.08	0.25 ± 0.03	0.80 ± 0.14	0.87 ± 0.12
Summit Lake	0.61 ± 0.11	0.49 ± 0.17	0.26 ± 0.07	0.46 ± 0.14	0.56 ± 0.24
Roadside Lake	0.70 ± 0.05	0.51 ± 0.14	0.50 ± 0.14	5.0 ± 3.9	0.78 ± 0.15
Smeaton Pond	2.6 ± 1.3	0.22 ± 0.05	0.15 ± 0.03	0.88 ± 0.29	0.78 ± 0.27

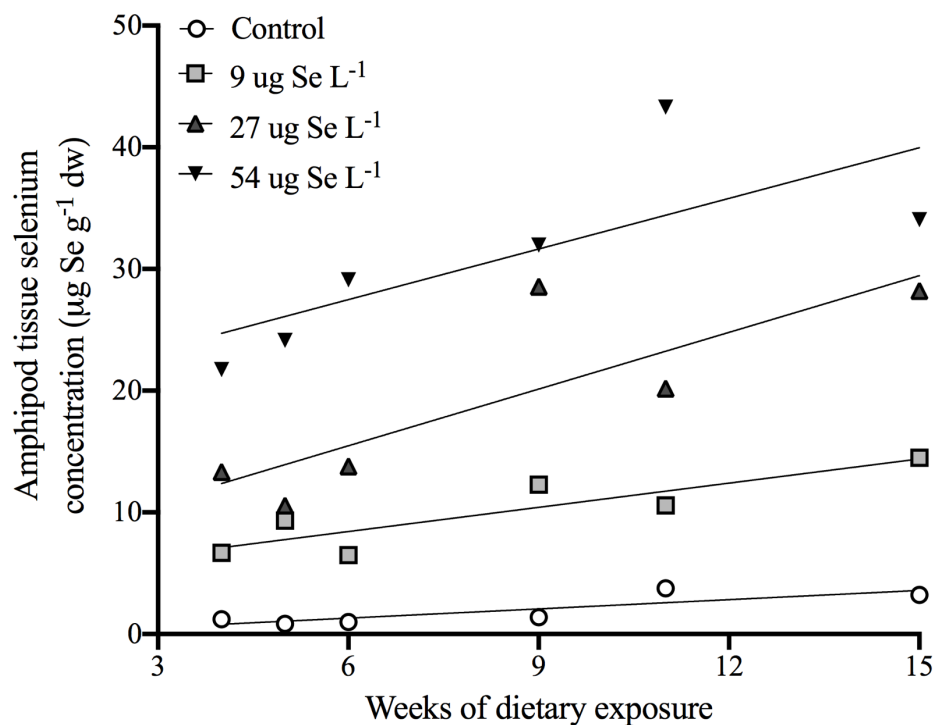


Figure A.3.1. Total *H. azteca* tissue Se concentration ($\mu\text{g Se g}^{-1} \text{ dw}$) during dietary treatment to Se. Treatments are defined as aqueous Se concentrations exposed to dietary alga. The four treatment groups were Control (white circle), 9 $\mu\text{g Se L}^{-1}$ (grey square), 27 $\mu\text{g Se L}^{-1}$ (grey upright triangle), and 54 $\mu\text{g Se L}^{-1}$ (black downward triangle). Lines represents the linear best fit of each respective treatment group.

Table A.3.1. Summary of mean (\pm SE) general water quality parameters and aqueous Se concentrations measured throughout laboratory exposures to respective dietary Se treatments.

Dietary Treatment	Temperature (°C)	Dissolved Oxygen (%)	pH	Conductivity ($\mu\text{S cm}^{-1}$)	Ammonia^a (mg L⁻¹)	Alkalinity (mg CaCO₃ L⁻¹)	Hardness (mg CaCO₃ L⁻¹)	Se ($\mu\text{g L}^{-1}$)
Control (1.6 $\mu\text{g Se g}^{-1}$ dw)	27 \pm 0.4	93 \pm 1.5	7.9 \pm 0.03	475 \pm 1	BDL	133 \pm 1	146 \pm 2	0.34 \pm 0.01
Low SeIV (6.9 $\mu\text{g Se g}^{-1}$ dw)	27 \pm 0.4	93 \pm 2.0	8.0 \pm 0.03	477 \pm 1	BDL	134 \pm 2	145 \pm 1	0.36 \pm 0.01
High SeIV (19 $\mu\text{g Se g}^{-1}$ dw)	27 \pm 0.4	94 \pm 1.3	7.9 \pm 0.02	477 \pm 1	BDL	135 \pm 1	146 \pm 1	0.38 \pm 0.01

^a Below detection limit (BDL): detection limit = 0.25 mg L⁻¹

Table A.3.2. Mean (\pm SE) developmental days (100% eye up and 100% hatch) and survival at swim up (%) of F1 larval *P. promelas* fertilized by parental generation exposed to three treatments of dietary Se (Control [$1.6 \mu\text{g Se g}^{-1} \text{ dw}$]), Low SeIV [$6.9 \mu\text{g Se g}^{-1} \text{ dw}$], and High SeIV [$19 \mu\text{g Se g}^{-1} \text{ dw}$]).

Dietary Treatment	Days to 100% Eye Up	Days to 100% Hatch	Survival at Swim Up (%)
Control	2.0 ± 0.0	5.1 ± 0.2	86 ± 4.5
Low SeIV	2.0 ± 0.0	5.6 ± 0.3	93 ± 2.5
High SeIV	2.2 ± 0.2	5.2 ± 0.2	95 ± 3.2

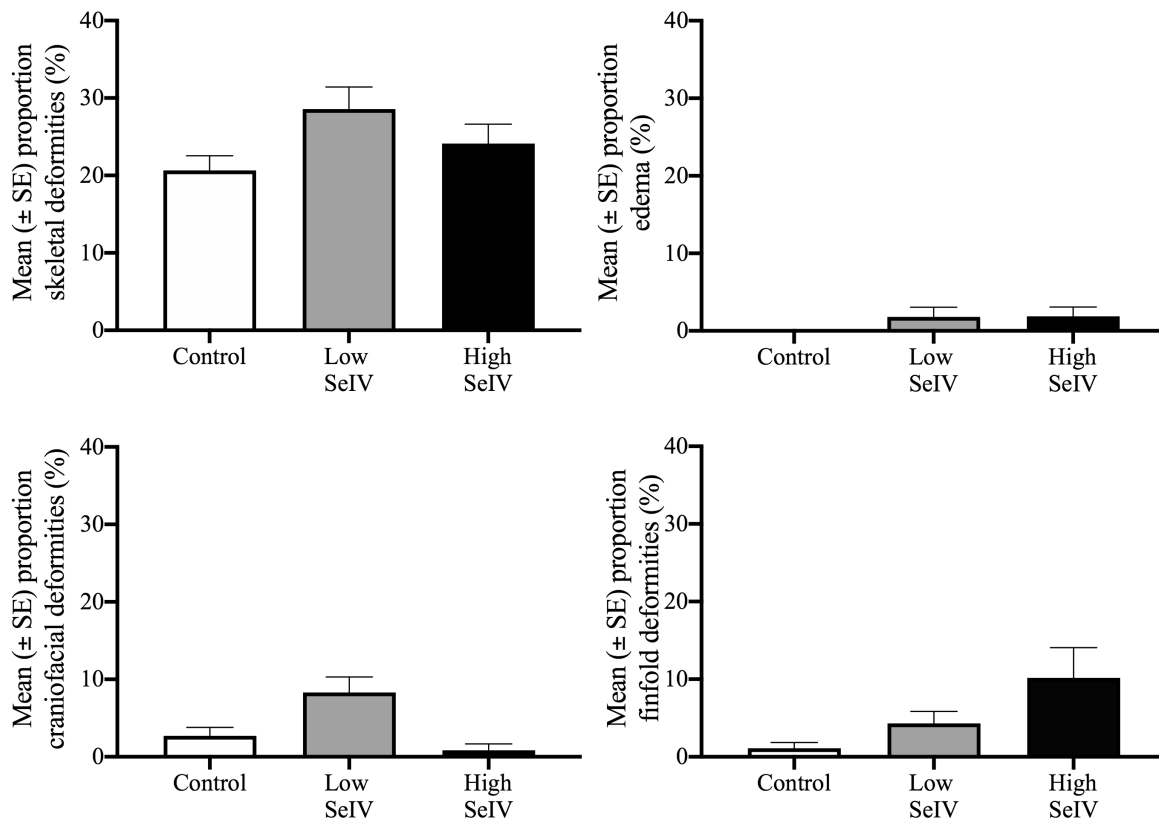


Figure A.3.2. Mean (\pm SE) deformities (skeletal, edema, craniofacial, and finfold) present at swim up in F1 larval *P. promelas* fertilized by parental generation exposed to three treatments of dietary Se (Control [$1.6 \mu\text{g Se g}^{-1} \text{dw}$]), Low SeIV [$6.9 \mu\text{g Se g}^{-1} \text{dw}$], and High SeIV [$19 \mu\text{g Se g}^{-1} \text{dw}$]).